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THE THEORETICAL VALUES OF CORRELATIONS BETWEEN RELATIVES IN RANDOM MATING POPULATIONS[1]*

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Received June 3, 1954

THE problem which will be discussed in this paper is the partition of variance in a random mating diploid population and the use of that partition in determining the theoretical values of the covariances and correlations between relatives. The paper will contain a description of the partition of variance which holds for the case when there are an arbitrary number of unlinked loci and an arbitrary number of alleles at each locus. This partition is sufficiently general to enable exact specification of the covariances and correlations between relatives in the population, providing there is no inbreeding in the relatives considered. Here the words "no inbreeding" are used in the sense that there is zero probability, that any two alleles in any individual are both copies of a single gene in the ancestry. No discussion will be given in this paper of the case when there is inbreeding.

The results to be described on correlations between relatives may be regarded as the extension of previous work in the literature indicated by table 1.

The correct results for the case of one locus can be readily extended by the obvious broadening of definitions to the case of an arbitrary number of loci providing there is no epistacy. Instead of the term epistacy some workers use the term interactions between loci, because of the historical associations of the term epistacy. The following is an example of the existence of epistacy for the case of two loci, with genes A, a and B, b respectively, the figures in the body of the table denoting the "true" observed characteristic.

The essential point is that the change in characteristic resulting from a change with respect to the A locus varies with the actual state at the B locus at which the change takes place: Thus

$$AA\ BB - Aa\ BB = 9 - 7 = 2$$

$$AA\ Bb - Aa\ Bb = 6 - 5 = 1$$

$$AA\ bb - Aa\ bb = 1 - 2 = -1$$

and so on. Actually the epistacy exhibited here is between two loci or factors, and can be exhibited by making an analysis of variance of the following form.

Source	*d.f.*
Between A genotypes	2
Between B genotypes	2
Interaction	4
Total	8

* Part of the cost of formulae and tables has been paid by the GALTON AND MENDEL MEMORIAL FUND.

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TABLE 1

Tabular summary of work on correlations between relatives

Alleles	Degree of dominance	Locus effects	System of mating	Relationship	Author, year reference	Notes
$\frac{1}{2}A + \frac{1}{2}a$	Complete	additive	random	ancestral fraternal	Pearson, 1904	No environmental effects
$\frac{1}{2}A + \frac{1}{2}a$	any	additive	random	ancestral	Yule, 1906	
Any no. any p's	any (?*)	additive	random	ancestral fraternal	Weinberg, 1908, 1910	
Any no. any p's	complete	2 factor multiplicative	random	ancestral fraternal	Weinberg, 1910	
Any no. any p's	any	2 factor general	random	ancestral fraternal uncle nephew cousins	Fisher, 1918	
Any no. any p's	any	additive small effects	assortative equilibrium	ancestral fraternal	Fisher, 1918	
Any no. any p's	none (any in certain cases)	additive	any inbreeding	any	Wright, 1921, 1922, 1951	
Any no. any p's	none (any in certain cases)	additive	any inbreeding	any	Wright, 1933, 1951	Sex-linkage
Any no. any p's	none (all loci) complete (all loci)	additive on square root of distance from optimum	random	parent - offspring full-sib	Wright, 1935, 1952	Also with environment additive on primary scale
Any no. any p's	any	additive	any inbreeding	any	Malécot, 1948	Solution indicated only

* Weinberg (1908, *1* p. 447) found that if the parent offspring correlation is $\frac{1}{2}(1 - k)$ then the full-sib correlation is $\frac{1}{2}\left(1 - \frac{k}{2}\right)$ for the case of a single locus (or loci with additive effects) with no environmental effects. He also found ancestral correlations. His manipulations with dominance (1908 *2* pp. 313–317) are peculiar and do not cover the case of arbitrary dominance relationships since he supposes the heterozygote to be always at a definite point between the two corresponding homozygotes.

TABLE 2

Example of epistacy

	Genotype at first locus		
	AA	*Aa*	*aa*
Genotype at second locus			
BB	9	7	6
Bb	6	5	4
bb	1	2	4

The analysis of variance for a 3 x 3 factorial experiment with two factors, *a* and *b*, each with levels 0, 1, and 2 is well known and the complete breakdown given in standard statistical texts is of interest because it foreshadows the general structure. This analysis of variance is given in table 3. It should be noted that there will be proportional frequencies in the genetic case and not equal frequencies as in the usual designed experiment. The linear effect of factor *a* will be found to correspond to the additive effect of gene *A*: the quadratic effect of factor *a* corresponds to the average dominance at the *A* locus, and likewise for the factor *b*. The subdivision of the inter-

TABLE 3

Analysis of variance of a 3 x 3 factorial experiment

	Source	d.f.
Factor *a*	linear (A′)	1
	quadratic (A″)	1
Factor *b*	linear (B′)	1
	quadratic (B″)	1
Interaction	A′ x B′	1
	A′ x B″	1
	A″ x B′	1
	A″ x B″	1
Total		8

action into linear x linear, linear x quadratic, quadratic x linear, and quadratic x quadratic components suggests that a valuable breakdown of the epistatic two-loci interactions should exist. This will be seen later to be the case. The particular example given in table 2 is intended to have no special features, such as the existence of one type of interaction and not another.

The present paper will contain rules by which the covariances under panmixia (and hence correlations) between any pair of relatives may be derived for a character involving any number of loci, any number of alleles, any dominance and any epistatic effects which exist in the population.

The derivation of the results in this paper is contained in another paper (Kempthorne 1954). The aim of the present paper is to present the results in a form suitable for the user of genetic statistics together with some discussion of interesting particular cases.

THE PARTITION OF TOTAL VARIANCE IN THE POPULATION

Only the case in which the effects of environment and genotype are additive will be discussed. In that case we may write the simple equation

$$P = G + E$$

where: P is the phenotypic value (which is a number) of the individual with the particular genotype;

G is the average phenotypic value which would be observed if a large number of individuals with the particular genotype are grown in a particular class of environments at random and is called the genotypic value:

E is an environmental deviation arising because the genotype is grown in a particular environment, rather than over the whole class of environments. By definition G and E are uncorrelated, and if both G and E are measured from the population mean both have an average value or expectation of zero. In a real genetic population G and E may not be uncorrelated, and their effects may not be additive. A number of extra problems arise. In the present situation, we may write

$$\sigma_P^2 = \sigma_G^2 + \sigma_E^2$$

where
σ_P^2 is the phenotypic (observed) variance,
σ_G^2 is the genotypic variance, or variance between true phenotypic values,
σ_E^2 is the environmental variance.

Now we turn to the decomposition of σ_G^2 into components. It is shown in the cited paper that

$$\sigma_G^2 = \sigma_A^2 + \sigma_D^2 + \sigma_{AA}^2 + \sigma_{AD}^2 + \sigma_{DD}^2 + \sigma_{AAA}^2 + \sigma_{AAD}^2 + \sigma_{ADD}^2 + \sigma_{DDD}^2$$

and so on; where
σ_A^2 is the variance of the total additively genetic values;
σ_D^2 is the variance of the total dominance contributions;
σ_{AA}^2 is the variance of the epistatic contributions of the type additive x additive from all pairs of loci;
σ_{AD}^2 is the variance of the epistatic contributions of the type additive x dominance from all pairs of loci and so on;
σ_{AAA}^2 is the variance of the epistatic contributions of the type additive x additive x additive from all sets of three loci;
σ_{AAD}^2 is the variance of the epistatic contributions of the type additive x additive x dominance from all sets of three loci;
σ_{ADD}^2 is the variance of the epistatic contributions of the type additive x dominance x dominance from all sets of three loci and so on.
When there are a total of n loci involved, there will be terms like σ_x^2, where x contains all combinations of rA's and sD's, r plus s being less than or equal to n.

The meaning of each term in the expansion of σ_G^2 is as follows. The additively genetic variance, σ_A^2 arises as the sum of squares of a quantity for each locus. The quantity for a locus say the A locus, is obtained by classifying the population according to the A locus and getting the mean genotypic value for each genotype according to the A locus. If there are three alleles at this locus, there are six genotypes: A_1A_1, A_1A_2, A_1A_3, A_2A_2, A_2A_3, A_3A_3. Let the frequencies of these genotypes and the mean genotypic values be as follows:

Genotype	A_1A_1	A_1A_2	A_1A_3	A_2A_2	A_2A_3	A_3A_3
Frequency	p_1^2	$2p_1p_2$	$2p_1p_3$	p_2^2	$2p_2p_3$	p_3^2
Genotypic value	y_{11}	y_{12}	y_{13}	y_{22}	y_{23}	y_{33}

Let the overall mean genotypic value be zero; in any particular case one merely subtracts the population mean of the genotypic values to achieve this. Then the average effects of the genes A_1, A_2 and A_3 are found to be:

Gene	*Average effects*
A_1	$p_1y_{11} + p_2y_{12} + p_3y_{13} = \alpha_1$, say
A_2	$p_1y_{12} + p_2y_{22} + p_3y_{23} = \alpha_2$, say
A_3	$p_1y_{13} + p_2y_{23} + p_3y_{33} = \alpha_3$, say.

Then the additively genetic variance arising from this locus is equal to

$$2p_1\alpha_1^2 + 2p_2\alpha_2^2 + 2p_3\alpha_3^2 .$$

We may do the same operation for each locus, and add the resulting quantities, to give the additively genetic variance, σ_A^2, in the population. We may note in passing

that the contribution to the total additively genetic value of an individual arising from the A locus is equal to $\alpha_i + \alpha_j$ for the individual with genotype A_iA_j.

To define the dominance variance we again consider the A locus, and given the average effects we can immediately construct the dominance deviation for each genotype arising from the A locus by means of the equation:

$$d_{ij} = y_{ij} - \alpha_i - \alpha_j$$

where d_{ij} is the dominance deviation arising from the A locus for the individual of genotype A_iA_j at the A locus. The dominance variance arising from this locus is then equal to

$$p_1^2d_{11}^2 + 2p_1p_2d_{12}^2 + 2p_1p_3d_{13}^2 + p_2^2d_{22} + 2p_2p_3d_{23}^2 + p_2^2d_{33}^3.$$

The total dominance deviation of an individual is the sum of the dominance deviations arising from each of the loci, and the total dominance variance is the sum of the dominance variance contributions arising from each of the loci. It is important in the further description to note that the dominance deviation for the A_iA_j individual arising from the A locus can be written formally as

$$d_{ij} = \{A_i - \sum p_sA_s\}\ \{A_j - \sum p_sA_s\}$$

in which the whole expression on the right hand side is expanded and then the genotypic values are inserted for each genotype. In general with m alleles at a locus the summation inside the curly brackets extends from 1 to m.

To describe the epistatic contributions arising from two loci, we take a particular pair of loci, say A and B loci. Let the gene frequencies at the B locus be denoted by q, with subscripts. We can denote the genotype with respect to these two loci by $A_iA_jB_kB_l$, and the mean genotypic values by y_{ijkl}. The additive x additive epistatic contribution from this pair of loci, for the individual with genotype $A_iA_jB_kB_l$ is obtained by writing down the formal expression

$$(\alpha\alpha)_{ik} = (A_i - \sum p_sA_s)(\sum p_sA_s)(B_k - \sum q_tB_t)(\sum q_tB_t)$$

and the terms $(\alpha\alpha)_{il}$, $(\alpha\alpha)_{jk}$ and $(\alpha\alpha)_{jl}$, which are obtained by making the necessary substitutions. Each of the expressions $(\alpha\alpha)_{ik}$ etc., must be expanded and then the genotypic value for each genotype inserted and the whole expression reduced to a single number. In a certain sense there are four additive x additive contributions for each genotype. These four contributions will be uncorrelated in the population and have the same variance. The additive x additive variance arising from the A and B loci is equal to four times the mean value over the population of $[(\alpha\alpha)_{ik}]^2$. The total additive x additive contribution to a genotypic value for any individual in the original n-locus population is equal to the sum of 4 terms from each pair of loci, so that there are $4\,\frac{n(n-1)}{2}$ parts in all. The additive x additive variance is the sum of the additive x additive variances arising from each pair of loci.

The additive x dominance epistatic contribution for the individual with genotype $A_iA_jB_kB_l$ is obtained by writing down the formal expression:

$$(\alpha d)_{ikl} = \{A_i - \sum p_sA_s\}\{\sum p_sA_s\}(B_k - \sum q_tB_t)(B_l - \sum q_tB_t)$$

and the terms $(\alpha d)_{jkl}$, $(d\alpha)_{ijk}$, $(d\alpha)_{ijl}$. Each of these expressions $(\alpha d)_{ikl}$ etc. must be expanded and then the mean genotypic value inserted for each genotype and the whole expression reduced to a single number. There are then 4 additive x dominance contributions arising from a particular pair of loci. These four contributions are uncorrelated. The additive x dominance variance arising from the A and B loci is equal to the sum of the mean values over the population of

$$[(\alpha d)_{ikl}]^2,\ [(\alpha d)_{ijl}]^2,\ [(d\alpha)_{iik}]^2 \text{ and } [(d\alpha)_{ijl}]^2.$$

As before we add over all pairs of loci to get either the total additive x dominance contribution to the genotypic value or the genotypic variance.

To shorten the presentation we shall now exhibit two examples of formal expressions which must be expanded to give the contributions to mean genotypic values. The contributions to genotypic variance are obtained by averaging the squares of the resulting numbers over all possible combinations of alleles and then over all possible combinations of loci.

Dominance x dominance: One contribution per genotype per pair of loci:

$$(dd)_{ijkl} = [A_i - \sum p_u A_u][A_j - \sum p_u A_u][B_k - \sum q_t B_t][B_k - \sum q_t B_t]$$

Additive x additive x additive: eight contributions per genotype for each set of three loci: let the loci be denoted by A, B and C, with gene frequencies denoted by p, q, r with subscripts:

$$(\alpha\alpha\alpha)_{ijk} = [A_i - \sum p_u A_u][\sum p_u A_u][B_j - \sum q_t B_t][\sum q_t B_t][C_k - \sum r_u C_u][\sum r_u C_u]$$

The general rules for writing down these formal expressions are as follows: for each time the word "additive" occurs in the name of the epistatic contribution we have a factor like

$$[A_i - \sum p_u A_u][\sum p_u A_u]$$

and for each time the word "dominance" occurs in the name of the epistatic contribution we have a factor like

$$[B_k - \sum q_t B_t][B_l - \sum q_t B_t].$$

All the necessary factors are written down, the resulting expression expanded and mean genotypic values inserted.

The method of description given above was the way in which the breakdown of the genotype value was derived. It is of use in indicating the structure of the effects, dominance and epistatic deviations, because for instance a factor like

$$A_i - \sum p_u A_u$$

has an average equal to zero, since

$$\begin{aligned} &\sum p_i [A_i - \sum p_u A_u] \\ &= \sum p_i A_i - \sum p_u A_u \\ &= 0 \end{aligned}$$

There is however a much simpler way of representing all the terms, which will be exemplified for the case of two loci. It will be remembered that the y_{ikl} have an average value of zero. Now we define quantities like $y_{i\cdots}$, $y_{ij\cdot\cdot}$, $y_{i\cdot k\cdot}$, $y_{\cdot kl}$ etc., following a fairly common statistical notation,

$$y_{i\cdots} = \sum_{j,k,l} p_j q_k q_l y_{ijkl}$$

$$y_{ij\cdot\cdot} = \sum_{k,l} q_k q_l y_{ijkl}$$

$$y_{i\cdot k\cdot} = \sum_{j,l} p_j q_l y_{ijkl}$$

$$y_{i\cdot kl} = \sum_{j} p_j y_{ijkl}$$

The general idea is that we are taking averages. The particular subscripts over which averaging has been done are replaced by a dot. It should be noted that the averages are taken weighted according to gene frequency. So, for example, when the subscript i is replaced by a dot, we multiply by p_i and sum over the range of i.

In the case of two loci, we may represent y_{ikl} by the following equation

$$y_{ijkl} = \alpha_i^1 + \alpha_j^1 + d_{ij}^1 + \alpha_k^2 + \alpha_l^2 + d_{kl}^2 + (\alpha^1\alpha^2)_{ik} + (\alpha^1\alpha^2)_{jk} + (\alpha^1\alpha^2)_{il} + (\alpha^1\alpha^2)_{jl} + (\alpha^1 d^2)_{ikl} + (\alpha^1 d^2)_{jkl} + (d^1\alpha^2)_{ijk} + (d^1\alpha^2)_{ijl} + (d^1 d^2)_{ijkl},$$

in which the superscript represents the locus (*not* a power), so that for example α_k^2 is the average effect of the k-th allele at locus 2. We note also that $y_{ijkl} = y_{jikl} = y_{ijlk} = y_{jilk}$ with similar relationships if any subscripts are replaced by a dot. We then have the following expressions;

$$\alpha_i^1 = y_{i\cdots}, \alpha_j^1 = y_{\cdot j\cdot\cdot},$$

$$d_{ij}^1 = y_{ij\cdot\cdot} - y_{i\cdots} - y_{\cdot j\cdot\cdot},$$

$$\alpha_k^2 = y_{\cdot\cdot k\cdot}, \alpha_l^2 = y_{\cdots l},$$

$$d_{kl}^2 = y_{\cdot\cdot kl} - y_{\cdot\cdot k\cdot} - y_{\cdots l},$$

$$(\alpha^1\alpha^2)_{ik} = y_{i\cdot k\cdot} - y_{i\cdots} - y_{\cdot\cdot k\cdot} = y_{i\cdot k\cdot} - \alpha_i^1 - \alpha_k^2,$$

$$(\alpha^1\alpha^2)_{il} = y_{i\cdot\cdot l} - y_{i\cdots} - y_{\cdots l} = y_{\cdots l} - \alpha_i^1 - \alpha_l^2,$$

$$(\alpha^1\alpha^2)_{jk} = y_{\cdot jk\cdot} - y_{\cdot j\cdot\cdot} - y_{\cdot\cdot k\cdot} = y_{\cdot jk\cdot} - \alpha_j^1 - \alpha_k^2,$$

$$(\alpha^1\alpha^2)_{jl} = y_{\cdot j\cdot l} - y_{\cdot j\cdot\cdot} - y_{\cdots l} = y_{\cdot j\cdot l} - \alpha_j^1 - \alpha_l^2,$$

$$(\alpha^1 d^2)_{ikl} = y_{i\cdot kl} - \alpha_i^1 - \alpha_k^2 - \alpha_l^2 - d_{kl}^2 - (\alpha^1\alpha^2)_{ik} - (\alpha^1\alpha^2)_{il},$$

$$(\alpha^1 d^2)_{jkl} = y_{\cdot jkl} - \alpha_j^1 - \alpha_k^2 - \alpha_l^2 - d_{kl}^2 - (\alpha^1\alpha^2)_{jk} - (\alpha^1\alpha^2)_{jl},$$

$$(d^1\alpha^2)_{ijk} = y_{ijk\cdot} - \alpha_i^1 - \alpha_j^1 - d_{ij}^1 - \alpha_k^2 - (\alpha^1\alpha^2)_{ik} - (\alpha^1\alpha^2)_{jk},$$

$$(d^1\alpha^2)_{ijl} = y_{ij\cdot l} - \alpha_i^1 - \alpha_j^1 - d_{ij}^1 - \alpha_l^2 - (\alpha^1\alpha^2)_{il} - (\alpha^1\alpha^2)_{jl},$$

$$(d^1 d^2)_{ijkl} = y_{ijkl} - \alpha_i^1 - \alpha_j^1 - d_{ij}^1 - \alpha_k^2 - \alpha_l^2 - d_{kl}^2 - (\alpha^1\alpha^2)_{ik} - (\alpha^1\alpha^2)_{il} - (\alpha^1\alpha^2)_{jk} - (\alpha^1\alpha^2)_{jl} - (\alpha^1 d^2)_{ikl} - (\alpha^1 d^2)_{jkl} - (d^1\alpha^2)_{ijk} - (d^1\alpha^2)_{ijl}.$$

TABLE 4

Types and numbers of contributions to genotypic value

Type of contribution	*Number of contributions*
additive	2n
dominance	n
additive x additive (AA)	$4\frac{n(n-1)}{2}$
additive x dominance (AD)	$4\frac{n(n-1)}{2}$
dominance x dominance (DD)	$\frac{n(n-1)}{2}$
additive x additive x additive (AAA)	$8\frac{n(n-1)(n-2)}{6}$
additive x additive x dominance (AAD)	$12\frac{n(n-1)(n-2)}{6}$
additive x dominance x dominance (ADD)	$6\frac{n(n-1)(n-2)}{6}$
dominance x dominance x dominance (DDD)	$\frac{n(n-1)}{6}\frac{(n-2)}{}$

It is perhaps of interest to record in table 4 the total number of contributions of the simpler types to the genotypic value of an individual.

It is not necessary to evaluate each possible effect and deviation and form the sum of squares of each type to get the variance contributions. It is simpler to note that these may be obtained from the sum of squares of the averages indicated above. We shall again exemplify the process by the two locus case. It is necessary to identify the loci to which the component of variance belongs. We therefore use the following notation:

$\sigma^2_{A_1}$ = additive genetic variance due to locus 1
$\sigma^2_{D_1}$ = dominance variance due to locus 1
$\sigma^2_{A_2}$ = additive genetic variance due to locus 2, etc.
$\sigma^2_{A_1A_2}$ = additive x additive variance due to loci 1 and 2
$\sigma^2_{A_1D_2}$ = additive x dominance variance due to loci 1 and 2

We then find

$$\sum_i p_i y^2_{i\cdots} = \tfrac{1}{2}\sigma^2_{A_1},$$

$$\sum_{ij} p_i p_j y^2_{ij\cdots} = \sigma^2_{A_1} + \sigma^2_{D_1},$$

$$\sum_{ik} p_i q_k y^2_{i\cdot k\cdot} = \tfrac{1}{2}\sigma^2_{A_1} + \tfrac{1}{2}\sigma^2_{A_2} + \tfrac{1}{4}\sigma^2_{A_1A_2},$$

$$\sum_{ijk} p_i p_j q_k y^2_{ijk\cdot} = \sigma^2_{A_1} + \sigma^2_{D_1} + \tfrac{1}{2}\sigma^2_{A_2} + \tfrac{1}{2}\sigma^2_{A_1A_2} + \tfrac{1}{4}\sigma^2_{A_2D_1},$$

$$\sum_{ikl} p_i q_k q_l y^2_{i\cdot kl} = \tfrac{1}{2}\sigma^2_{A_1} + \sigma^2_{A_2} + \sigma^2_{D_2} + \tfrac{1}{2}\sigma^2_{A_1A_2} + \tfrac{1}{4}\sigma^2_{A_1D_2},$$

$$\sum_{ijkl} p_i p_j q_k q_l y^2_{ijkl} = \sigma^2_{A_1} + \sigma^2_{D_1} + \sigma^2_{A_2} + \sigma^2_{D_2} + \sigma^2_{A_1A_2} + \sigma^2_{A_1D_2} + \sigma^2_{A_2D_1} + \sigma^2_{D_2D_2}.$$

The multipliers to the components are written down by noting the number of deviations of a particular type arising in the averages being squared and summed. Thus $y_{ijk.}$ contains (1) two average effects at locus one, each contributing $\frac{1}{2}\sigma^2_{A_1}$ (2) the dominance deviation at locus one contributing $\sigma^2_{D_1}$, (3) one average effect at locus two contributing $\frac{1}{2}\sigma^2_{A_2}$, (4) two additive x additive deviations each contributing $\frac{1}{4}\sigma^2_{A_1A_2}$, (5) one additive x dominance deviation contributing $\frac{1}{4}\sigma^2_{A_2D_1}$. The total variance components are then,

$$\sigma^2_A = \sigma^2_{A_1} + \sigma^2_{A_2}$$

$$\sigma^2_D = \sigma^2_{D_1} + \sigma^2_{D_2}$$

$$\sigma^2_{AD} = \sigma^2_{A_1D_2} + \sigma^2_{A_2D_1}$$

The generalizations to the case of more than two loci are easily written down.

THE COVARIANCE OF RELATIVES

The covariance between two relatives, there being no inbreeding, can be written down from the knowledge of two quantities, which following MALÉCOT (1948) we denote by ϕ and ϕ'. Let us suppose for purposes of definiteness, that the two relatives are related because the genes they each receive from their sires can be traced back to a certain set of genes possessed by one or more common ancestors, and likewise for the genes they receive from their dams. Let the genes at one locus which could be given to the two relatives by way of their sires be denoted by a, b, c, d, etc. Then we can calculate merely by looking at the pedigrees, the probability that both relatives receive gene a by way of their sires. Let this probability be added over all the possible sire genes and denote the result by ϕ. Do likewise with the dam genes and let the result be ϕ'.

In the more general case when the sire genes and dam genes are not distinct sets, we may regard one individual X as having genes x_s, x_d at a locus and the related individual Y as having genes y_s, y_d at that locus. We must then use

$$\frac{\phi + \phi'}{2} = \frac{P(x_s = y_s) + P(x_s = y_d) + P(x_d = y_s) + P(x_d = y_d)}{2},$$

$$\phi\phi' = P(x_s = y_s, x_d = y_d) + P(x_s = y_d, x_d = y_s),$$

where $P(x_s = y_s)$ is the probability that x_s and y_s are identical by descent and so on.

The basic result is that the covariance of the relatives with respect to a variance component σ^2_z where z contains rA's and sD's is equal to

$$\left[\frac{(\phi + \phi')}{2}\right]^r \cdot [\phi\phi']^s.$$

Examples of the use of the result

Parent-offspring. Let the parent be the sire, and suppose the genes at one locus are a and b. Then the probability that both parent and offspring contain the gene a is $1/2$ and likewise for gene b it is $1/2$. So ϕ is equal to unity. The dam genes of the offspring are taken at random from the population, so that ϕ' is equal to zero.

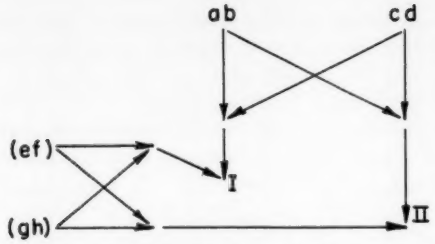

FIGURE 1. A pedigree showing double first cousins

Hence we have

$$Cov(P, O) = \tfrac{1}{2}\sigma_A^2 + \tfrac{1}{4}\sigma_{AA}^2 + \tfrac{1}{8}\sigma_{AAA}^2 + \tfrac{1}{16}\sigma_{AAAA}^2 + \text{etc.}$$

Full sibs. Let the sire genes be a and b and the dam genes be a' and b'. Then the probability that both offspring receive a is 1/4 and likewise for b, so that ϕ is equal to 1/2. Similarly ϕ' is equal to 1/2. Hence

$$Cov(F. S) = \tfrac{1}{2}\sigma_A^2 + \tfrac{1}{4}\sigma_D^2 + \tfrac{1}{4}\sigma_{AA}^2 + \tfrac{1}{8}\sigma_{AD}^2 + \tfrac{1}{16}\sigma_{DD}^2 + \tfrac{1}{8}\sigma_{AAA}^2 + \tfrac{1}{16}\sigma_{AAD}^2 + \tfrac{1}{32}\sigma_{ADD}^2 + \tfrac{1}{64}\sigma_{DDD}^2 + \text{etc.}$$

Double first cousins. Let the mating be represented by figure 1. The probability that gene a reaches both individual I and individual II is equal to 1/16, and likewise for genes b, c, d. So ϕ equal to 1/4. Similarly ϕ is equal to 1/4, and consequently

$$\frac{(\phi + \phi')}{2} = \frac{1}{4}$$

$$\phi\phi' = \frac{1}{16}$$

Hence the covariance is equal to

$$\tfrac{1}{4}\sigma_A^2 + \tfrac{1}{16}\sigma_D^2 + \tfrac{1}{16}\sigma_{AA}^2 + \tfrac{1}{64}\sigma_{AD}^2 + \tfrac{1}{256}\sigma_{DD}^2 + \tfrac{1}{64}\sigma_{AAA}^2 + \tfrac{1}{256}\sigma_{AAD}^2 + \tfrac{1}{1024}\sigma_{ADD}^2 + \tfrac{1}{4096}\sigma_{DDD}^2 + \text{etc.}$$

CORRELATIONS BETWEEN RELATIVES

Under the circumstances specified in the earlier parts of this paper, we see immediately that the correlation between relatives is equal to

$$\frac{\text{Covariance}}{\sigma_G^2 + \sigma_E^2}$$

If there is no selection in the population this will also be the regression of the one relative or the other.

DISCUSSION OF RESULTS

For the degrees of relationship which will occur in the human population, and probably in any biological population the values of ϕ and ϕ' will be such that the

TABLE 5

Analysis of variance

Source	d.f.	Mean square	Expected mean square
Between sires	s-1	S	$\sigma_3^2 + p\sigma_2^2 + pd\sigma_3^2$
Between dams within sires	$s(d$-1)	D	$\sigma_1^2 + p\sigma_2^2$
Between progeny	$sd(p$-1)	P	σ_1^2
Total	sdp-1		

epistatic contributions involving three or more loci will be negligible, even if the corresponding variance components in the original population are a large proportion of the total genotypic variance. It is of interest however to note that, for example, if we had a population in which the total phenotypic variance were wholly genotypic so that σ_E^2 is equal to zero and this genotypic variance where wholly additive x additive x additive, then the correlation between parent and offspring would be 0.125.

APPLICATION OF BASIC RESULT

For purposes of getting an idea about the heritability of an attribute in a population, a common procedure is to mate each of s sires to a random sample of sd dams, d dams for each sire, and obtain p progeny from each mating. The analysis of variance given in table 5 is then computed. The expected mean squares are obtainable from the basic result given in the paper as follows.

Between progeny mean square. This is the average of half of the squared differences of progeny within dams, i.e. $\frac{1}{2}E(x_{ijk} - x_{ijk'})^2$ where i, j denote the sire and dam respectively and k and k' the two progeny, and x_{ijk} is the value of the characteristic measured from the population mean. Now this is equal to

$$\tfrac{1}{2}\{E(x_{ijk}^2) + E(x_{ijk'}^2) - 2E(x_{ijk}x_{ijk'})\},$$

and we have under the model of additive environmental and genotypic effects:

$$E(x_{ijk}^2) = E(x_{ijk'}^2) = \sigma_P^2$$

and

$$E(x_{ijk}x_{ijk'}) = \text{covariance between full sibs} = Cov(F \cdot S)$$

Hence the expected mean square is

$$\sigma_1^2 = \sigma_P^2 - Cov(F \cdot S)$$

Between dams within sires mean square. This is equal to $p/2$ times the average value of the squared difference of dam means within the same sire, i.e. is equal to

$$\frac{p}{2} E(x_{ij\cdot} - x_{ij'\cdot})^2$$

or

$$\frac{1}{2p} E\{\sum_k x_{ijk} - \sum_k x_{ij'k}\}^2$$

If we expand this and take expectations we find

$$\frac{1}{2p}\{2p\sigma_P^2 + 2p(p-1)\,Cov(F.S) - 2p^2\,Cov(H,S)\}$$

where

$$Cov(H.S) = \text{covariance of half sibs.}$$

Hence we find that the expected value of the mean square is equal to

$$[\sigma_P^2 - Cov(F.S)] + p[Cov(F.S) - Cov(H.S)]$$

Between sires mean square. This is equal to $pd/2$ times the average value of the squared difference of sire means, i.e. is equal to

$$\frac{pd}{2}E(x_{i..} - x_{i'..}),$$

or

$$\frac{2}{pd}E\{\sum_{jk} x_{ijk} - \sum_{jk} x_{i'jk}\}^2,$$

or

$$\frac{1}{pd}E(\sum_{jk} x_{ijk})^2$$

since the sires are mated to different dams. The quantity $(\sum_{jk} x_{ijk})^2$ may be expanded as $\sum_j (\sum_k x_{ijk})^2 + \sum_{j\neq j'} (\sum_k x_{ijk})(\sum_k x_{ij'k})$,
and

$$(\sum_k x_{ijk})^2 \text{ leads to } p\sigma_P^2 + p(p-1)\,Cov(F.S),$$

$$(\sum_k x_{ijk})(\sum_k {}_{ij'k}) \text{ leads to } p^2\,Cov(H.S),$$

so that the final result is

$$\frac{1}{pd}[dp\sigma_P^2 + dp(p-1)Cov(F.S) + d(d-1)p^2Cov(H.S)]$$

$$= \sigma_P^2 + (p-1)Cov(F.S) + p(d-1)Cov(H.S)$$

$$= \sigma_P^2 - Cov(F.S)] + p[Cov(F.S) - Cov(H.S)] + pd\,Cov(H.S)$$

We therefore have:

$$\sigma_1^2 = \sigma_P^2 - Cov(F.S)$$

$$\sigma_2^2 = Cov(F.S) - Cov(H.S)$$

$$\sigma_3^2 = Cov(H.S).$$

Consequently $\sigma_1^2 + \sigma_2^2 + \sigma_3^2$ is the denominator of heritability, being equal to σ_P^2. It has been suggested in the literature that $4\hat{\sigma}_3^2$ or $4\hat{\sigma}_2^2$ may be used in the numerator,

TABLE 6

Genotypic values for worked example

	A_1A_1			A_1A_2			A_2A_2		
	B_1B_1	B_1B_2	B_2B_2	B_1B_1	B_1B_2	B_2B_2	B_1B_1	B_1B_2	B_2B_2
C_1C_1	0	2	1	1	1	3	2	4	5
C_1C_2	0	2	3	0	4	5	3	5	6
C_2C_2	0	3	4	3	4	6	2	4	5

where hats denote estimates obtained by equating observed mean squares to expected mean squares and solving.

The estimate

$$\hat{h}^2 = \frac{4\hat{\sigma}_3^2}{\hat{\sigma}_1^2 + \hat{\sigma}_2^2 + \hat{\sigma}_3^2}$$

is a consistent estimate of

$$\frac{1}{\sigma_P^2}\{\sigma_A^2 + \tfrac{1}{4}\sigma_{AA}^2 + \tfrac{1}{16}\sigma_{AAA}^2 + \text{etc.}\}$$

The estimate

$$\hat{h}^2 = \frac{4\hat{\sigma}_2^2}{\hat{\sigma}_1^2 + \hat{\sigma}_2^2 + \hat{\sigma}_3^2}$$

is a consistent estimate of

$$\frac{1}{\sigma_P^2}\{\sigma_A^2 + \sigma_D^2 + \tfrac{3}{4}\sigma_{AA}^2 + \tfrac{1}{2}\sigma_{AD}^2 + \tfrac{1}{4}\sigma_{DD}^2 + \tfrac{7}{16}\sigma_{AAA}^2 + \text{etc.}\}$$

We note therefore that $\hat{h}^2$ is only mildly sensitive to non-additive effects of genes, the numerator involving 1/4 of one component of two-factor interaction variance and one-eight of one component of three factor interaction variance plus terms presumably of lower order. The estimate $\hat{h}^2$ is valid only if the effects of the genes are additive, and any type of deviation from additivity will cause the estimate to be inconsistent.

There are of course difficulties, such as the existence of maternal effects, which complicate the matter. These will not be discussed here.

A WORKED EXAMPLE

For the purpose of illustrating the results given earlier, it was thought worthwhile to work out an example of the partition of variance in a population with 3 loci. This population was chosen arbitrarily with 2 genes at each locus, denoted by A_1, A_2 and B_1, B_2 and C_1, C_2. The gene frequencies chosen for A_1, B_1, and C_1 were 0.3, 0.4, and 0.5 respectively. The genotypic values were taken to be those in table 6. These genotypic values were chosen to exemplify various possibilities: for example the gene substitution C_2 in place of C_1 has no effect when the a and b loci are in phases A_1A_1 and B_1B_1; when the a locus is in phase A_2A_2, the C locus exhibits over-

dominance regardless of the phase of the b locus. It was found that the components of genotypic variance are as follows:

$$\sigma_A^2 = 1.965 \qquad \sigma_{AD}^2 = 0.158 \qquad \sigma_{AAD}^2 = 0.002$$
$$\sigma_D^2 = 0.189 \qquad \sigma_{DD}^2 = 0.049 \qquad \sigma_{ADD}^2 = 0.040$$
$$\sigma_{AA}^2 = 0.143 \qquad \sigma_{AAA}^2 = 0.011 \qquad \sigma_{DDD}^2 = 0.059$$

The proportions of variance are as follows:

Additive	75.1%
Dominance	7.2
Epistatic	17.7
	100.0%

The covariances between relatives are not wildly different from what would have been guessed ignoring epistacy. For instance the true parent offspring covariance is

$$\tfrac{1}{2}(1.965) + \tfrac{1}{4}(0.143) + \tfrac{1}{8}(0.002) = 1.018.$$

whereas the covariance guessed by ignoring epistacy is 0.982. The purpose of this example is not however to indicate that the preceding statement holds in general, but merely to indicate that all the details can be worked out in any specified case.

SUMMARY

The general solution to the problem of the correlations between relatives in a random mating diploid population is described. The basic result is that the genotypic variance σ_G^2 can be partitioned into components according to the equation

$$\sigma_G^2 = \sigma_A^2 + \sigma_D^2 + \sigma_{AA}^2 + \sigma_{AD}^2 + \sigma_{DD}^2 + \sigma_{AAA}^2 + \sigma_{AAD}^2 + \sigma_{ADD}^2 + \sigma_{DDD}^2 + \text{etc.}$$

The different components on the right-hand side are defined. The covariance between relatives is stated in terms of these components. Some simple illustrations of the use of the result in obtaining correlations are given together with an application of the results. The partition of variance is computed and presented for a chosen arbitrary set of genotypic values.

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THE CORRELATION BETWEEN RELATIVES IN A SIMPLE AUTOTETRAPLOID POPULATION[1]*

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IT is well known that the simple autotetraploid population

$$p^4A^4 + 4p^3qA^3a + 6p^2q^2A^2a^2 + 4pq^3Aa^3 + q^4a^4$$

is in equilibrium under random mating. The problem to be considered herein is that of the correlation between relatives in such a population. The assumption is that segregation is by chromosomes rather than chromatids; that is, if an individual is *abcd*, then there are six equally likely contributions to the offspring consisting of *ab*, *ac*, *ad*, *bc*, *bd*, and *cd*. We suppose that the phenotype (P) of an autotetraploid is made up additively of two parts which are uncorrelated, a genotypic value (G) and an environmental error (E). It will be supposed that relatives are correlated only because their genotypic values are correlated and that there is no correlation between their environmental errors. We shall consider the case when only one locus is involved, and there are m alleles involved, say A_i with frequencies p_i, $i = 1, 2, \cdots, m$ The equilibrium population may be specified briefly by its genotypic array:

$$\{\sum p_iA_i\}^4$$

The results given here are an extension of those of SEWALL WRIGHT (1938), who considered the case when the genes are additive in their effects.

THE BREAKDOWN OF GENOTYPIC VALUE

Consider an individual with genotype $A_iA_jA_kA_l$. Then we have the identity

$$\begin{aligned}A_iA_jA_kA_l = {} & \rho^4 + (A_i - \rho)\rho^3 + \rho(A_j - \rho)\rho^2 + (A_i - \rho)(A_j - \rho)\rho^2 + \rho^2(A_k - \rho)\rho \\ & + (A_i - \rho)\rho(A_k - \rho)\rho + \rho(A_j - \rho)(A_k - \rho)\rho + (A_i - \rho)(A_j - \rho)(A_k - \rho)\rho \\ & + \rho^3(A_l - \rho) + (A_i - \rho)\rho^2(A_l - \rho) + \rho(A_j - \rho)\rho(A_l - \rho) \\ & + (A_i - \rho)(A_j - \rho)\rho(A_l - \rho) + \rho^2(A_k - \rho)(A_l - \rho) + (A_i - \rho)\rho(A_k - \rho)(A_l - \rho) \\ & + \rho(A_j - \rho)(A_k - \rho)(A_l - \rho) + (A_i - \rho)(A_j - \rho)(A_k - \rho)(A_l - \rho). \qquad (1)\end{aligned}$$

where ρ is equal to $\sum_{i=1}^{m} p_iA_i$. Now let us expand each of the terms on the right hand side, and replace each genotype symbol by the genotypic value of that genotype. Using $A_iA_jA_kA_l$ also to denote the genotypic value of the genotype $A_iA_jA_kA_l$ on the left hand side we may write the result as

$$\begin{aligned}A_iA_jA_kA_l = {} & \mu + \alpha_i + \alpha_j + \beta_{ij} + \alpha_k + \beta_{ik} + \beta_{jk} + \gamma_{ijk} + \alpha_l \\ & + \beta_{il} + \beta_{jl} + \gamma_{ijl} + \beta_{kl} + \gamma_{ikl} + \gamma_{ikl} + \delta_{ijkl}, \qquad (2)\end{aligned}$$

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where the terms are made to correspond one by one, and for instance μ is the population mean, α_i is equal to the expansion of $(A_i - \rho)\rho^3$ expressed in genotypic values, that is, it is equal to the following expression in genotypic values:

$$\sum_a p_a^3 A_i A_a^3 + 3\sum_{a \neq b} p_a^2 p_b A_i A_a^2 A_b + \sum_{a \neq b \neq c} p_a p_b p_c A_i A_a A_b A_c \text{ minus } \mu$$

and so on.

We now state that the terms on the right-hand side of (2) have zero means and are uncorrelated in the population, that is: if we suppose that a random assignment of the numbers 1, 2, 3, 4 is made to the genes of each individual in the population, so as to give the genes an order, then the covariance of each term on the right hand side between two random individuals is zero. The fact that the means are zero is obvious. A couple of examples are sufficient to exhibit orthogonality of terms. Consider the terms α_i and $\alpha_{i'}$ for 2 random individuals: then for each, i and i' take the values a and $a' = 1, 2, \cdots, m$ with probability p_a and $p_{a'}$ independently. Hence

$$\begin{aligned} E(\alpha_i \alpha_{i'}) &= \sum_a p_a[(A_a - \rho)\rho^3] \sum_{a'} p_{a'}[(A_{a'} - \rho)\rho^3] \\ &= (\rho^4 - \rho^4)(\rho^4 - \rho^4) \\ &= 0. \end{aligned}$$

Again take β_{ik} for one individual and γ_{ikl} for another. We have

$$\begin{aligned} \underset{i,k,l}{E}(\beta_{ik}\gamma_{ikl}) &= \underset{ik}{E}\,\beta_{ik}\{\underset{l}{E}\,\gamma_{ikl}\} \\ &= \underset{ik}{E}\,\beta_{ik}\{0\} \\ &= 0. \end{aligned}$$

We see therefore that the genotypic value of an individual tetraploid is made up additively of the following parts:

(1) μ the population mean;

(2) four gene effects α_i, α_j, α_k, α_l which by analogy with diploid population may be called the additive effects of genes A_i, A_j, A_k and A_l;

(3) six terms arising from the interaction of two genes, β_{ij}, β_{ik}, β_{ik}, β_{il}, β_{jl}, β_{kl}, which are analogous to dominance deviations in the diploid case;

(4) four terms arising from the interaction of three genes, γ_{ijk}, γ_{ijl}, γ_{jkl} and γ_{ikl}, to which again there is no analogy in the diploid case;

(5) One term arising from the interaction of four genes, δ_{ijkl}, to which again there is no analogy in the diploid case.

The fact that the α_j's are the additively genetic effects of the genes A_i follows from the definition of additive genetic effects and the orthogonality or lack of correlation between the terms on the right-hand side of (2).

There appears to be no terminology established for terms like the γ's or δ's defined above. One might perhaps suggest that terms like β_{ij} should be called digene effects, terms like γ_{ijk} trigene effects, and terms like δ_{ijkl} quadrigene effects.

The values of the different contributions are exhibited in explicit form below.

Definitions:

$$y_{ijkl} = \text{genotypic value as deviation from population mean.}$$

$$\overline{A_iA_jA_kA_l} = y_{ijkl}$$

$$\overline{A_iA_jA_k} = \sum_a p_l y_{ijka}$$

$$\overline{A_iA_j} = \sum_a \sum_b p_a p_b y_{ijab}$$

$$\overline{A_i} = \sum_a \sum_b \sum_c p_a p_b p_c y_{iabc}$$

Contributions:

$$\alpha_i = \overline{A_i}, \alpha_j = \overline{A_j}, \alpha_k = \overline{A_k}, \alpha_l = \overline{A_l}$$

$$\beta_{ij} = \overline{A_iA_j} - \alpha_i - \alpha_j$$

$$\beta_{ik} = \overline{A_iA_k} - \alpha_i - \alpha_k$$

$$\beta_{il} = \overline{A_iA_l} - \alpha_i - \alpha_l$$

$$\beta_{jk} = \overline{A_jA_k} - \alpha_j - \alpha_k$$

$$\beta_{jl} = \overline{A_jA_l} - \alpha_j - \alpha_l$$

$$\beta_{kl} = \overline{A_kA_l} - \alpha_k - \alpha_l$$

$$\gamma_{ijk} = \overline{A_iA_jA_k} - \beta_{ij} - \beta_{ik} - \beta_{jk} - \alpha_i - \alpha_j - \alpha_k$$

$$\gamma_{ijl} = \overline{A_iA_jA_l} - \beta_{ij} - \beta_{il} - \beta_{jl} - \alpha_i - \alpha_j - \alpha_l$$

$$\gamma_{ikl} = \overline{A_iA_kA_l} - \beta_{ik} - \beta_{il} - \beta_{kl} - \alpha_i - \alpha_k - \alpha_l$$

$$\gamma_{jkl} = \overline{A_jA_kA_l} - \beta_{jk} - \beta_{jl} - \beta_{kl} - \alpha_j - \alpha_k - \alpha_l$$

$$\delta_{ijkl} = \overline{A_iA_jA_kA_l} - \gamma_{ijk} - \gamma_{ijl} - \gamma_{ikl} - \gamma_{jkl} - \beta_{ij} - \beta_{ik} - \beta_{il} - \beta_{jk} - \beta_{jl} - \beta_{kl} - \alpha_i - \alpha_j - \alpha_k - \alpha_l$$

Finally we can envisage the partition of genotypic variance by the equation

$$\sigma_G^2 = \sigma_A^2 + \sigma_D^2 + \sigma_T^2 + \sigma_F^2$$

where

$$\tfrac{1}{4}\sigma_A^2 = \sum_{l=l}^{m} p_i \alpha_i^2$$

$$\tfrac{1}{6}\sigma_D^2 = \sum_{i,j} p_i p_j \beta_{ij}^2$$

$$\tfrac{1}{4}\sigma_T^2 = \sum_{i,j,k} p_i p_j p_k \gamma_{ijk}^2$$

and

$$\sigma_I^2 = \sum_{ijkl} p_i p_j p_k p_l \delta_{ijkl}^2 .$$

These components of variance will be found useful in characterising correlations between relatives.

THE CORRELATIONS BETWEEN RELATIVES

Because of the assumption

$$P = G + E$$

where E is a random variable completely independent from genotype and from relative to relative, the correlation of two individuals say I and II reduces to

$$\frac{Cov(G_I , G_{II})}{\sigma_P^2} .$$

Hence we have to evaluate the covariances between relatives.

We shall make the assumption that the two individuals are related because the sire genes of both come from some set of genes in the population and the dam genes come from another set of genes in the population which is independent of the former set. There is assumed to be no inbreeding in the pedigree, so that the genes on the sire side are statistically independent of the genes on the dam side. Also it will be assumed that there is no inbreeding in the ancestries leading to either sire genes or dam genes, so that the frequency with which the sire genes are any particular ordered pair say A_i and A_j is equal to $p_i p_j$ the frequency in the original population. The same assumption is made about the dam genes.

Let the genes A_i , A_j , A_k , A_l of any individual be ordered so that genes A_i and A_j are genes contributed by the sire of the individual and genes A_k and A_l are contributed by the dam.

Let A_{s1} , A_{s2} , $\cdots$, A_{sk} be the set of genes which can be sire genes of the two relatives. These are a random set of the totality of possible genes. If one relative receives A_{s1} say and another does not receive A_{s1} then the covariance between the two relatives with respect to additive effects is zero. The covariance will be equal to $\frac{1}{4}\sigma_A^2$, if both relatives receive A_{s1} . Let p_{s1}^I be the probability that the first relative receives gene A_{s1} and p_{s1}^{II} the probability that the second relative receives gene A_{s1} . Then the covariance between the relatives because of their both inheriting gene A_{s1} is

$$P_{s1}^I P_{s1}^{II} \{\text{additive effect of } A_{s1}\}^2$$

which on the average will be equal to $P_{s1}^I P_{s1}^{II} \frac{1}{4}\sigma_A^2$.
Now let

$$\phi = \sum_{\substack{\text{sire} \\ \text{alleles}}} P_S^I P_S^{II} ,$$

then the covariance in additively genetic variance because of sire genes is equal to $\phi(\frac{1}{4}\sigma_A^2)$. Let ϕ' be defined similarly for the dam genes. Then the total covariance with respect to the additive gene effects is

$$(\phi + \phi')\tfrac{1}{4}\sigma_A^2$$

When we come to the digenic effects we have to calculate the sum of the probabilities that any pair of original genes are inherited by both relatives on the sire side. Let this be ψ and let the corresponding number of the dam side be ψ'. Also we need the sum of the probabilities that the same gene will be inherited on the sire side and the same gene on the dam side. This is equal to $\phi\phi'$. Then the covariance with respect to what we have called digenic effects is equal to

$$[\phi\phi' + \psi + \psi']\frac{\sigma_D^2}{6}$$

In the case of trigenic effects we need the sum of the probabilities that (1) both relatives receive the same pair of genes from their sires and only one gene in common from their dams, or (2) vice versa. This is easily seen to be

$$(\phi\psi' + \phi'\psi),$$

and the covariance is equal to

$$(\phi\psi' + \phi'\psi)\frac{\sigma_T^2}{4}$$

Finally in the case of quadrigenic effects the covariance is equal to

$$\psi\psi'\sigma_F^2 .$$

Combining all the terms we have the result that the covariance between the relatives is equal to

$$(\phi + \phi')\tfrac{1}{4}\sigma_A^2 + [\phi\phi' + \psi + \psi']\tfrac{1}{6}\sigma_D^2 + (\phi\psi' + \phi'\psi)\tfrac{1}{4}\sigma_T^2 + \psi\psi'\sigma_F^2$$

In cases when the sire and dam genes are not distinct sets, the multipliers of σ_A^2, σ_D^2, σ_T^2, σ_F^2 are analogous quantities relating to the probabilities that genes of the two relatives are identical by descent.

EXAMPLES OF THE USE OF THE FORMULA

(1) *Parent-offspring*

We have

$$\phi = (\tfrac{1}{2} + \tfrac{1}{2} + \tfrac{1}{2} + \tfrac{1}{2}) = 2,$$
$$\phi' = 0$$
$$\psi = (\tfrac{1}{6} + \tfrac{1}{6} + \tfrac{1}{6} + \tfrac{1}{6} + \tfrac{1}{6} + \tfrac{1}{6}) = 1,$$
$$\psi' = 0.$$

Hence the covariance is equal to

$$\tfrac{1}{2}\sigma_A^2 + \tfrac{1}{6}\sigma_D^2$$

(2) *Full-sib*
We have

$$\phi = \tfrac{1}{4} \times 4 = 1,$$
$$\phi' = \tfrac{1}{4} \times 4 = 1,$$
$$\psi = \tfrac{1}{36} \times 6 = \tfrac{1}{6},$$
$$\psi' = \tfrac{1}{36} \times 6 = \tfrac{1}{6},$$

so that the covariance is equal to

$$\tfrac{1}{2}\sigma_A^2 + \tfrac{2}{9}\sigma_D^2 + \tfrac{1}{12}\sigma_T^2 + \tfrac{1}{36}\sigma_F^2 .$$

(3) *Double first cousin*
We find

$$\phi = \tfrac{1}{2} = \phi'$$
$$\psi = \frac{8}{6^3} = \psi',$$

so that the covariance is equal to

$$\tfrac{1}{4}\sigma_A^2 + \frac{70}{6^4}\sigma_D^2 + \frac{2}{6^3}\sigma_T^2 + \frac{64}{6^6}\sigma_F^2 .$$

(4) *Parent-grandoffspring*
Here we are

$$\phi = 1, \phi' = 0,$$
$$\psi = \tfrac{1}{6}, \psi' = 0,$$

so that the covariance is equal to

$$\tfrac{1}{4}\sigma_A^2 + \tfrac{1}{36}\sigma_D^2$$

NOTES ON THE RESULTS

The results given for the four types of relatives enable the components σ_A^2, σ_D^2, σ_T^2 and σ_F^2 to be estimated by equating observed covariances to expected covariances, care having been taken to eliminate environmental correlations. Since in the original population under the assumptions made

$$\sigma^2 = \sigma_G^2 + \sigma_E^2$$
$$= \sigma_A^2 + \sigma_D^2 + \sigma_T^2 + \sigma_F^2 + \sigma_E^2,$$

we may also estimate σ_E^2. If one wishes one may then estimate ratios of the components, in the same way as ratios in the diploid case are calculated to estimate what are termed heritabilities.

It is of interest to note that if one had a character which is determined by one locus apart from an additive effect of environment one could use the formulae given

here and the corresponding well known ones for the diploid case to determine whether the inheritance of that character was diploid or autotetraploid.

The formulae given may be extended with no change in symbolism to deal with the case when we have an arbitrary number of loci among which there is no linkage and also no epistacy.

The present treatment may be extended to the case of an arbitrary number of independently segregating loci. This will be reserved for a later note, although we remark that the epistatic components now consist of a large number of types: in the case of interactions between two loci there are 10 distinct types of epistatic variance which enter differently into covariances between relatives.

The extension to higher degrees of polyploidy will be worked out by the same methodology.

SUMMARY

The correlations between relatives in a single-locus autotetraploid population in which there is no inbreeding are expressed by a simple formula involving numbers which can be calculated when one knows the chain of relationship of the relatives. Some notes on the results are given.

ACKNOWLEDGMENT

I am indebted to Dr. Sewall Wright for suggestions about presentation.

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GENETIC ANALYSIS OF INTERSPECIFIC HYBRIDS IN THE GENUS BROMUS, SECTION CERATOCHLOA

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SPECIES of the Ceratochloa section of Bromus, with somatic chromosome numbers of 42 were hybridized by G. L. STEBBINS during the period 1943 to 1945. This was done to study the relationships between the species and to test the agricultural usefulness of the hybrids. In 1947 I began an investigation of these diploid and allopolyploid hybrids, attempting to explain the underlying cytological and genetic causes of their partial sterility. The parental material consisted of strains of three species described below:

Bromus catharticus Vahl.

B. catharticus is generally a large-leaved vigorous species with large spikelets and very short awns. The lemmas are 14 to 18 or 20 mm long and 11-nerved; they are heavily scabrous along the keel and along the smaller nerves but the surface of the lemma between the nerves is glabrous or nearly so. The leaves, about 30 cm long, are glabrous, but the basal and lower sheath in this strain is densely pubescent. The species is common in, and probably native to, southern Brazil, Uruguay and northeastern Argentina, but it has been introduced as a forage grass wherever the climate is suitable. The strain used was from the Waite Research Institute, Australia.

Bromus haenkeanus Presl.

B. haenkeanus Presl. is less luxuriant than *B. catharticus*. Its leaves are narrower and approximately half the length, or about 12 cm. The spikelets are much narrower. The lemmas are from 9 to 14 mm long and are 7- to 9-nerved. The awns in the strains used were approximately 1 mm long. The lemma is uniformly, but not densely, scabrous. The species is typically an annual or a short-lived perennial like *B. catharticus*. It ranges through the Andes from Colombia to Patagonia. The two species occur together in several areas where they apparently remain quite distinct from each other. In North America *B. haenkeanus* has been introduced into California, Arizona, Nevada and Utah. I worked primarily with wild strains found near Carmel, California, and Sparks, Nevada.

Bromus stamineus Desv.

B. stamineus Desv. is a grass of moderate size, though slightly less vigorous and shorter-leaved than *B. catharticus*. The leaves are 12 cm, or more, in length, and both leaves and stems are rather densely pubescent. The lemmas are 12 to 15 mm long, 11-, 13-, or occasionally 15-nerved, and are frequently tinged with purple. The surface of the lemma is uniform in texture, but more densely scabrous than in *B. haenkeanus*. The awns are very long compared to those of the other two species, averaging

8 to 10 mm. This species is strongly perennial. In South America it is restricted to central Chile, but has been introduced in central California, where it has been found occasionally around Berkeley and in Sonoma County.

METHODS

Culture methods

The parental strains were grown side by side in the grass garden on the Oxford Tract at the University of California. The hybridizations were made in the field during the early summer of 1944. The F_1 seeds were germinated in a greenhouse and young plants were later transplanted to the field. These were allowed to flower and produce seed for the F_2 generation. The three species used in this work produce both chasmogamous and cleistogamous flowers; however, under the prevailing conditions of culture the flowers produced the first season were so regularly cleistogamous that no further treatment seemed necessary to insure self-fertilization. The F_2 plants were scored for fertility and the three most fertile individuals were selected. The seed from each was used to establish an F_3 population of approximately 20 plants. In a similar way, following selection for fertility, four new F_4 populations were propagated.

Other F_1 plants were divided, rooted in sand, and then treated with colchicine to induce polyploidy. From the tetraploid culms produced on the treated plants seed was collected to establish the "C_2" generations. Cleistogamy was again relied on to effect self-fertilization. The six most fertile C_2 plants were selected, and from each a population of about 20 C_3 plants was established. Selection was repeated and the lines were continued as with the diploids.

Morphological analysis

Variation in the hybrid populations was described in terms of the two characters in which the parental varieties differed most widely. In *B. catharticus* x *haenkeanus* the useful characters were texture of the lemma and lemma length. The variability in texture ranged from a completely glabrous condition of the surface to a dense covering of scabrous hairs. Scores were assigned as follows:

0 Surface of lemma glabrous, i.e. not more than 6 to 10 hairs on the surface between the nerves. May be scabrous on the larger nerves. Typical of *Bromus catharticus*.

EXPLANATORY NOTE

Figures 1 and 2 show a phenotypic history of an interspecific hybridization from the parental species to the F_4 (diploid) and the C_4 (tetraploid). Variation is expressed in figure 1 in terms of lemma texture (nearly glabrous to densely pubescent) and of lemma length, and in figure 2 by nerve number and awn length. The intersection of horizontal and vertical lines represents the phenotypic mean of a population. In addition, the phenotype of each F_2 and C_2 individual is indicated. The seed fertility of the F_2 and C_2 plant is indicated by a diagonal line attached to the symbol representing the individual phenotype. The relative length of the diagonal represents the degree of fertility, the 100% point being marked by a short dash at 90 degrees to the fertility line. The dotted lines indicate the lines of descent.

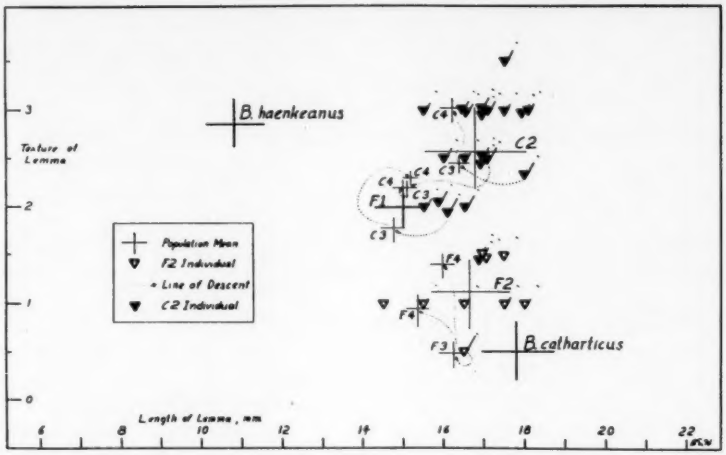

FIGURE 1. *Bromus catharticus* x *haenkeanus*. Phenotypes of parental species and of hybrid populations.

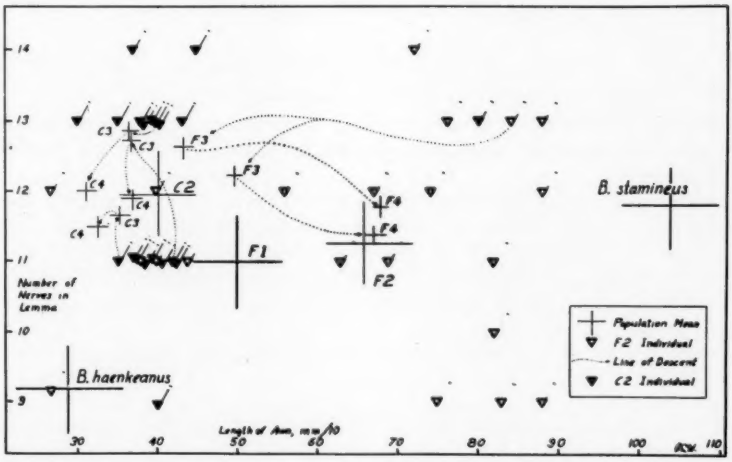

FIGURE 2. *Bromus haenkeanus* x *stamineus*. Phenotypes of parental species and of hybrid populations.

1 Surface very sparsely scabrous, only occasional scattered hairs present. Sometimes found in *catharticus*.

2 Lemma sparsely scabrous, distribution of hairs not uniform. Hairy patches alternating with bare patches. Typical of F_1 hybrid between *catharticus* and *haenkeanus*.

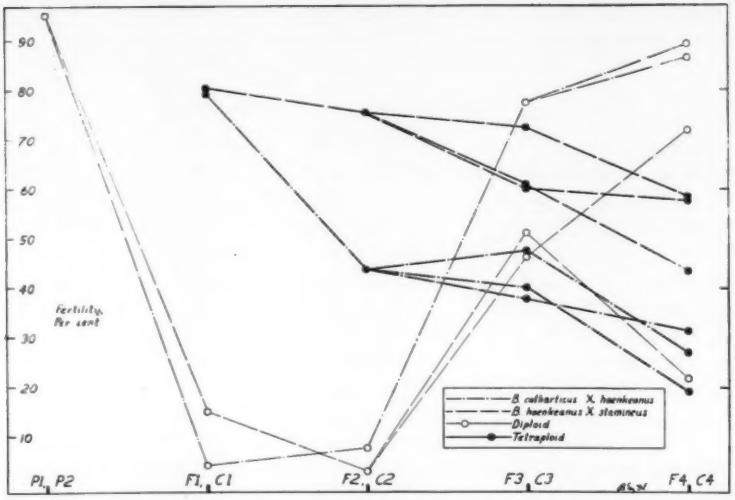

FIGURE 3. *Bromus catharticus*, *haenkeanus*, and *stamineus*. Average seed fertility of parental species, F_1 hybrids, and diploid and tetraploid lines of descent.

3 Surface of lemma covered uniformly and moderately densely with short, scabrous hairs. Typical of *Bromus haenkeanus*.

Lemma length was measured from the point of the callus to the tip of the lemma (not the awn). In *B. stamineus* x *haenkeanus* awn length and number of nerves in the lemma were analyzed.

The essential morphological data is presented in figures 1 and 2, using the scatter diagram technique of ANDERSON (1949). Each diagram shows the complete history of an interspecific hybridization to the 4th generation. The parental species, the F_1, F_3, and F_4 hybrids are represented by population means. In the F_2 (and C_2) generations, the phenotype of each individual plant is shown. The lines of descent may be traced by means of dotted lines.

Fertility

Fertility was determined by counting the fertile florets in nearly mature panicles. Florets were considered fertile if the caryopsis had developed to full size or nearly so by the time the flowering panicle had begun to turn brown. Estimations were based on samples of 50 or more florets per plant. The seed fertility of each F_2 and C_2 plant is indicated on the scatter diagrams (figures 1 and 2) by a diagonal line attached to the symbol representing the individual phenotype. The relative length of the diagonal indicates the degree of fertility, the 100% point is marked by a short dash at 90 degrees to the fertility line. The fertility indicated for the hybrid populations (figure 3) are averages based on samples of approximately 20 plants in each population.

Cytological analysis

For cytological analysis immature spikelets were fixed in a fresh solution of absolute alcohol and glacial acetic acid (3:1) for 24 hours and then transferred to 70% alcohol for storage. Temporary smears of pollen mother cells were stained with aceto-carmine. An alcohol vapor chamber technique was used (BRADLEY 1948) to make temporary smears permanent.

Statistical study

Did selection for fertility have any effect on the genotypes of the hybrid populations? Inspection of the scatter diagrams suggested a non-random relationship. The fertility of a hybrid seemed to depend on the degree of resemblance between the hybrid individual and its parent. The following test was devised: The radial distances $D_1, D_2, \ldots D_n$ were measured, on the scatter diagrams, from the mean phenotype of each hybrid population to the phenotype of each individual in the population. I assumed that the population mean is the best estimate of the genotype of the parent of the population. The quantities $S_1, S_2, \ldots S_n$ represent the seed fertility of each individual in the population, transformed from percentage to angular units according to the relation: angle = arc sin $\sqrt{\text{percentage}}$ (SNEDECOR 1946, p. 447). The correlation between fertility and segregation for morphological characters was then computed, using the product moment formula:

$$r = \frac{\sum sd}{\sqrt{\sum s^2 \sum d^2}}$$

RESULTS

Morphological

All the morphological data is presented in figures 1 and 2 for *catharticus* x *haenkeanus* and *haenkeanus* x *stamineus* respectively. Comparison of the 2nd to the 4th generations shows certain trends to be evident. A net decline in quantitative characters is typical of both the diploid and the tetraploid lines of descent. Lemma length consistently declines from F_2 to F_4 as in the C_2 to C_4 lines (figure 1). Awn length clearly decreases in the tetraploid lines of descent and seems to in the diploid. The number of nerves declines in the diploid lines and from C_3 to C_4 in all the tetraploids (figure 2).

The genotypes of the tetraploid lines seem to be effectively "fixed" at the C_2 level. In spite of considerable random variation, the phenotypes of the three tetraploid lines of descent from each F_1 hybrid maintain their relative positions from the C_2 to the C_4 generation.

Fertility

Seed fertility of the parental species is in excess of 90%. The F_1's *catharticus* x *haenkeanus* and *haenkeanus* x *stamineus* had a seed fertility of 4% and 15% respectively. This increased considerably in the later diploid generations; in the F_4 generation three out of the four diploid lines had approached the fertility of the original species. In the tetraploid material, the immediate effect of somatic doubling of the

TABLE 1

Correlation between segregation for length of lemma and texture of lemma, and segregation for fertility in the hybrid populations derived from the cross Bromus catharticus x haenkeanus

Population	N	r_{df}	Population	N	r_{df}
652B (F_2)	8	.615*	652C (C_2)	22	−.117
728 (F_2)	21	.446*	764A (C_3)	17	−.044
			764B (C_3)	18	.445*
Summary	29	.488†	764C (C_3)	25	.040
			809 (C_4)	9	−.552
808A (F_4)	16	.099	810ABC (C_4)	11	.198
808B (F_4)	14	−.42	811ABC (C_4)	17	−.458*
All diploid	59	.258*	Tetraploid	119	−.124

* Significant.
† Highly significant.

TABLE 2

Correlation between segregation for length of awn and number of nerves in lemma, and segregation for fertility in hybrid populations derived from the cross Bromus haenkeanus x stamineus

Population	N	r_{df}	Population	N	r_{df}
654 (F_2)	18	.235	655 (C_2)	21	−.417*
730 (F_2)	16	.155	718 (C_3)	20	−.105
731 (F_2)	13	.216	719 (C_3)	11	−.785†
			720 (C_3)	20	−.029
Summary	47	.188	818B (C_4)	13	−.174
			819 (C_4)	17	−.206
816A (F_4)	20	.0005			
817A (F_4)	17	.196			
All diploid	84	.144	Tetraploid	102	−.288†

* Significant.
† Highly significant.

F_1's was a marked increase to approximately 80%. These tetraploid plants have been designated the C_1 generation. From the high point of 80% the fertility of all the tetraploid lines declined more or less rapidly. By the C_4 generation the average of the *haenkeanus* x *stamineus* populations had declined to about 50%; the *catharticus* x *haenkeanus* hybrids had dropped to an average of 25% (see figure 3).

Correlations

The results of the correlation computations are indicated in tables 1 and 2. In the majority of cases the correlations between segregation for morphological characters and segregation for fertility are positive in the diploid populations, while the corresponding figures for the tetraploid groups are negative. Exceptions to the tendencies indicated are seen in the highly fertile F_4 populations. Population 817A, with an average fertility of only 22% has a fairly high positive correlation, while the other

TABLE 3

Chromosome behavior at metaphase I

Population	No. cells	Chromosome associations average per cell				Bivalents paired one end only
		IV	III	II	I	
Part I. *Bromus catharticus* x *haenkeanus*						
525 (F_1)	25	0.0	0.0	21.0	0.0	1.25
415 (F_1)	20	0.0	0.0	21.0	0.0	.90
766A (C_3)	19	7.84	0.58	24.95	0.79	—
766B (C_3)	10	7.40	0.10	27.00	0.10	—
Part II. *Bromus haenkeanus* x *stamineus*						
412 (F_1)	20	0.55	0.15	20.10	0.40	1.60
526 (F_1)	26	0.0	0.0	21.0	0.0	2.60
655 (C_2)	9	4.11	0.0	33.80	0.0	—
719 (C_3)	20	5.00	0.15	31.70	0.15	—
719* (C_3)	20	5.25	0.05	31.40	0.05	—

* Two different plants examined in this population.

F_4's, all with fertilities of 72% or more, had correlations of nearly zero or were negative. In the tetraploids there were three exceptional populations, all in the *catharticus* x *haenkeanus* series. The two with positive correlations of about .2 or more were in the same line of descent (764B and 810).

Cytological analysis

STEBBINS and TOBGY (1944) demonstrated that the chromosomes of the parental species formed bivalents only at metaphase I and behaved regularly during the later stages of meiosis. Of the four different interspecific hybrids examined cytologically, three formed 21 bivalents regularly at metaphase I. The fourth formed 20 bivalents and a ring of four chromosomes in 70% of the cells examined, the rest being normal (table 3). The number of open bivalents was somewhat greater in the *haenkeanus* x *stamineus* F_1 than in the other. The same F_1 showed more irregularities in the later stages of meiosis. The most striking difference between the two interspecific hybrids was seen in the frequency of micronuclei. Note that the *catharticus* x *haenkeanus* F_1, with only about 1.5% of tetrads with micronuclei, was the more sterile, while the other F_1's, with about 40% of the tetrads showing micronuclei, were fairly fertile.

The allotetraploids exhibit quadrivalent as well as bivalent chromosome association. Moreover, the frequency of micronuclei in the tetrad stage is higher than in the diploids. Average frequency of quadrivalents per cell varied from 4.1 to 7.8. The *catharticus* x *haenkeanus* plants produced, on the average, more quadrivalents than the *haenkeanus* x *stamineus* hybrids. The former tetraploids were fairly sterile while the latter were quite fertile. The cytological data is summarized in table 3 for metaphase I and table 4 for anaphases I and II and the tetrad stage. All the tetraploid plants analyzed cytologically at metaphase I had the expected chromosome number of 84.

TABLE 4

Chromosome behavior, anaphase I to tetrads

Population	Anaphase I			Anaphase II			Tetrads	
	No. cells	% with		No. cells	% with		No. cells	% with micronuclei
		Laggards	Bridges		Laggards	Bridges		
Part I. *Bromus catharticus* x *haenkeanus*								
525 (F_1)	53	3.8	0.0	22	0.0	0.0	71	1.4
415 (F_1)							65	1.5
766A (C_3)	70	67.1	1.4				66	60.6
766B (C_3)	50	76.0	0.0	30	46.7	40.0	63	33.3
Part II. *Bromus haenkeanus* x *stamineus*								
412 (F_1)	63	33.3	3.2				64	39.1
526 (F_1)	47	0.0	2.1	44	0.0	4.5	89	41.5
655 (C_2)	83	16.9	5.0					
655* (C_2)							27	7.4
719 (C_3)							59	71.2
719* (C_3)							67	58.2

* Two different plants examined in this population.

DISCUSSION

The principal facts established by the experiment were: (1) the sterility of the interspecific F_1 hybrids; (2) the increase in fertility of the diploid lines of descent; (3) the loss of fertility in the tetraploid lines of descent in spite of selection for fertility; (4) a correlation, positive in the diploid, negative in the tetraploid, between segregation for fertility and segregation for morphological characters.

Causes of sterility

DOBZHANSKY (1951) proposes two categories of hybrid sterility, genic and chromosomal. Genic sterility applies to the situation where the genetic constitution of the hybrid is such as to preclude its sexual maturity. Chromosomal sterility is sterility caused by lack of homology, or incomplete homology, between the chromosomes of the parents of a hybrid. This condition could give rise to inviable combinations following meiosis even though the hybrid constitution itself might be viable. The observed sterility in the F_1 hybrids described here apparently is not genic, the production of flowers and the cytological processes being normal up to the first metaphase of meiosis. Moreover, the fact that bivalent formation is regular excludes gross structural differences as the cause of sterility. Thus the sterility must be due to relatively slight differences between the parental sets of chromosomes. Since the two parents are different, although related, taxonomic species, the gene differences present in the hybrid may be sufficient to give rise to disharmonious and inviable recombinations. Likewise, structural differences may be present which are too small to affect the regularity of meiotic pairing but are large enough to give rise to lethal deficiencies in the gametophytes. The latter suggestion is recognized as the hypothesis of *cryptic*

structural hybridity (STEBBINS 1945). The criterion of somatic doubling (DOBZHANSKY 1951) has been offered as a means of distinguishing genic from chromosomal sterility: if the immediate effect of somatic doubling is a marked increase in fertility, then the sterility must have been due to differences in chromosome homology. Probable examples are *Primula kewensis* (NEWTON and PELLEW 1929), Raphanobrassica (KARPECHENKO 1928), Tradescantia (SKIRM 1942) and many others. On the other hand, if chromosome doubling fails to increase fertility, then sterility must have been at least partly genic. This situation has been observed in crosses between species of Lycopersicum (LESLEY and LESLEY 1943; MCARTHUR and CHIASSON 1947) and Solanum (PROPACH 1940; TATEBE 1936). The application of this criterion suggests that the sterility observed here was actually due to differences in homology between the chromosomes of the parents of the hybrid. It may not be possible to determine whether genic differences or a condition of structural hybridity causes the sterility of the F_1 hybrid. The following analysis will indicate that the observed results are consistent with the hypothesis of cryptic structural hybridity although they do not exclude the other possibility. The advantage of the structural hypothesis is that it readily lends itself to a quantitative analysis.

Diploid hybrids

Because of the indication that the sterility of the interspecific hybrids described here may be combinational in character, an analysis of hybrid meiosis from this point of view is appropriate. The diploid genetic complement of any two of the three parental species may be represented by the symbols AA and BB respectively; the complements of the respective gametophytes will then be A and B. The success of the parental species in the wild is evidence of the viability of the genetic combinations AA and BB. Likewise, the normally high seed and pollen fertility indicates viability of the haploid complements A and B. The vegetative vigor of the F_1 hybrids when grown in the field demonstrates that the new combination AB is also viable.

The gametophytes produced by the hybrid AB will contain various combinations of genetic material. Even in the event of normal bivalent formation in the F_1 hybrid, few, if any, of the gametophytes will contain material wholly from one or the other of the parental species.

Due to cytological crossing over, an A centromere may become associated with a mixture of A and B material. Assuming two chiasmata per bivalent (the minimum required to produce pairing at both ends of the bivalent) the probability of an A centromere with chromatin from the A parent only is $.5^2$ or .25. The probability of such an intact A chromatid segregating into any given spore is .5, and the probability, P, of the spore receiving any given number, N, of intact A chromatids is obtained from expanding $(p + q)^{21}$ where $p = .5^3 = .125$ and $q = 1 - p = .875$. Thus, for example, (table 5) the probability that a gametophyte would receive *any* six (out of 21 possible) intact A chromatids is 2.79% while the probability of receiving six or more is 3.91%. But the probability P′ of the spore receiving a number, N, of *designated* intact A chromatids is simply p^N. Thus, (table 5), only 3.81×10^{-4}% of the gametophytes will have any particular combination of six A chromatids in the intact condition. Probabilities less than 10^{-6} are not listed.

TABLE 5

Chromatid combinations in the gametophytes produced by an interspecific hybrid with 21 pairs of chromosomes

N	P	ΣP	P′
13	—	—	—
12	.0000013	.0000014	—
11	.0000108	.0000121	—
10	.0000756	.0000877	—
9	.000441	.000529	—
8	.00214	.00267	—
7	.00855	.0112	—
6	.0279	.0391	.00000381
5	.0733	.112	.0000305
4	.151	.263	.000244
3	.235	.498	.00195
2	.258	.756	.0156
1	.182	.938	.125
0	.0606	.999	

N = Number of intact chromatids (not involved in chiasmata) from a designated parental species, out of a possible 21.

P = Probability that any N intact chromatids will go to a spore.

ΣP = Summation of P.

P′ = Probability that N designated intact chromatids will go to a spore.

It is obvious that an F_1 hybrid is practically unable to produce a gametophyte with genetic material wholly from one or the other of the parental species. If viability of the gametophyte depends on the production of spores with a quantity of intact chromatin, then obviously a fairly small quantity of intact parental chromatin must be sufficient. The fertility, 4%, of the *catharticus-haenkeanus* hybrid corresponds to the frequency in table 5 of combinations containing about six or more intact chromatids of *catharticus* or *haenkeanus*. The *haenkeanus-stamineus* hybrid with a fertility of 15% must have had some viable gametophytes with as few as five intact chromatids.

The possibility of structural hybridity as a cause of F_1 sterility was mentioned earlier in this discussion. The concept of cryptic structural hybridity was first suggested by SAX (1933) and MÜNTZING (1938), while STEBBINS (1945) first proposed the term. A full discussion of this hypothesis of hybrid sterility is found in STEBBINS (1950). Briefly, the condition could originate through small segmental interchanges between non-homologous chromosomes or through overlapping inversions within a single chromosome. STEBBINS, VALENCIA and VALENCIA (1946) propose that the cytological effect of this condition would be lowered chiasma frequency and that the genetic effect would be sterility due to the deficiencies introduced into the gametophyte generation by crossing over between structurally different chromosomes. These writers suggest that the effect on fertility will be more pronounced than the change in chiasma frequency. They estimated that in diploid hybrids between grass species in the tribe Hordeae (n = 7) structural differences of as many as 30 small segments

may be present without altering the appearance of bivalent formation, but that more than four or five would be enough to produce complete sterility in the F_1's. Regular pairing combined with F_1 sterility has been previously observed in the genus Bromus (STEBBINS and TOBGY 1944). STEBBINS (1950) lists two intergeneric hybrids and 24 genera in which one or more interspecific hybrids showing this same behavior have been reported. Of these, seven were reported to have been tested by the method of artificial allopolyploidy. In five of those tested chromosomal sterility was confirmed by the regaining of fertility; in the remaining two (the closely related Solanum and Lycopersicum), genic sterility was indicated.

The importance of deficiencies as gametophyte lethals has been evaluated by the work of MCCLINTOCK (1938, 1941, 1943, 1944), also by CLARK and ANDERSON (1933), STADLER (1943), and STADLER and ROMAN (1948). In maize deficiencies in chromosomes 3, 5, 9, and 10 have been studied at pachytene where accurate measurements are possible. Their size varied from a maximum of 2.8% of the gametic chromatin down to the limit of visibility (less than 0.1% of the chromatin). Practically all deficiencies large enough to be visible at pachytene were lethal to the male gametophyte; the female gametophyte was less sensitive. None of the homozygous deficiencies were completely viable in the diploid tissues, where they appeared as chimeras. Inasmuch as the loss of less than 1% of the chromatin complement in maize is usually lethal, it is suggested that other plants may be similarly affected, although polyploid species should be less sensitive than diploids. The species of Bromus in the section Ceratochloa with 21 pairs of chromosomes are presumably polyploid since the basic number in the genus is seven. Since there are no known living species in this section having a lower chromosome number, it is probable that these species have not arisen recently. It might be expected, then, that they would be sensitive to deficiencies, but that, due to higher chromosome number and probable polyploid origin, they would be less sensitive than maize.

It is possible to estimate the degree of structural hybridity in the F_1 hybrids. Chromosomal differences may be either inversions or translocations or a combination of the two. If the differences are the result of inversions, the production of deficient gametophytes will depend on the frequency and position of crossing over in the F_1 hybrid. A "cryptic" condition would result from a successive overlapping inversion and reinversion (STEBBINS 1950, page 223). Designating the normal structure as ABCDEFGHIJ, an altered structure could be ABGCDEFHIJ. A chiasma between C and F in the F_1 hybrid would render both participating strands abnormal. The gametophyte receiving the strand deficient for G would presumably be abortive. The maximum abortion of gametophytes expected from this structural difference would thus be 25%, unless the distance between C and F were long enough for two or more chiasmata. Assuming that each structural difference behaved independently and that a gametophyte with one or more deficiencies is abortive, the minimum fertility in a hybrid with N structural differences of the kind described above would be $(.75)^N$.

If on the other hand the segmental differences should consist of small translocations, then sterility would be independent of chiasma frequency and position but would depend entirely on the distribution of chromosomes. Random distribution of two

TABLE 6

Estimation of number of structural differences in F_1 hybrids

Hybrid	Fertility	Number of segmental differences	
		Inversions	Translocations
catharticus x *haenkeanus*	4%	11 or more	5
haenkeanus x *stamineus*	15%	6 or more	3

pairs of chromosomes with a single reciprocal translocation would produce a maximum of 50% of abortive gametophytes. With N structural differences of this type segregating independently, the fertility would be equal to $(.5)^N$. In table 6 the number of cryptic structural differences in the F_1 hybrids is estimated according to these principles.

Selection for fertility in the diploids was highly effective in three generations (see figure 3). Since it seems likely that such sterility as appeared in the F_1 and subsequent generations was due to structural heterozygosity, the cause of increased fertility was apparently the selection of (structurally) homozygous lines.

In the relation between morphological variation and fertility in the diploid hybrids there is a tendency for the morphologically more extreme types to be more fertile. The hereditary cause of morphological variation in these hybrid populations must be segregation of the genes responsible for the differences between the parents. Therefore the observed relationship is probably due to linkage between factors determining morphological characters and those controlling the viability of the gametophyte. A model will clarify this point. Assume an interspecific hybrid with a basic chromosome number of n = 2. The partially homologous chromosomes A, a and B, b differ structurally as well as in genes affecting morphological characters. Thus the chromosomal constitution of the hybrid will be represented by the formula AaBb. With random distribution, the F_2 will contain the parental recombinations AABB and aabb. The F_1 combination will be present, together with new combinations such as AaBB, Aabb, aaBb, etc. The mean F_2 phenotype should approach that of the F_1. The structural heterozygote AaBb will be low in fertility and near the mean in phenotype. The parental types will have a maximum fertility and be most unlike the mean; the others should be intermediate both in fertility and morphological characters. Thus the hypothesis could readily account for the correlations observed.

A further point should be noted. After several generations of selfing the lines should approach homozygosity; as genetic variability decreases the correlations should also decline. Three of the F_4 populations (see table 1 and 2) exhibited correlations which were either negative or nearly zero. Inasmuch as the average fertility was very high, about 80%, the probable cause of the low correlation was that genetic variability had been nearly eliminated by selection. All the diploid populations of intermediate fertility showed the characteristic positive correlation.

Tetraploid hybrids

The behavior of the tetraploid derivatives of the colchicine-treated F_1 hybrids is more complex than that of the corresponding diploids. Somatic chromosome doubling

of the F_1 produces a C_1 plant which has two sets of chromosomes from each parental species. Since the sister sets derived by doubling are identical, pairing should take place between them. The partially homologous sets which pair in the diploid hybrid may also be expected to associate in the tetraploid. The fact that all the tetraploids show quadrivalent associations indicates that this is the case (table 3). According to WADDINGTON (1939), the pairing of the sister chromosomes produced by doubling would be termed homogenetic pairing, while the term heterogenetic pairing applies to the association of chromosomes of dissimilar origin as in the interspecific F_1's. The prevalence of bivalent association in an allotetraploid would suggest that pairing was homogenetic. On the other hand, the presence of quadrivalents is indication of heterogenetic association. The critical evidence in regard to the type of association lies in the variability of the tetraploid populations. It is obvious that the sister genomes derived from each parent must be genetically identical. Therefore there is no potential for genetic variability unless heterogenetic pairing takes place. Conversely any genetic variability in the tetraploids is evidence per se of heterogenetic pairing.

The rate at which this potential variability is set free will depend on two factors: (1) the relative frequency of homogenetic as against heterogenetic association in the tetraploid and (2) the statistical limitations on gene segregation in the tetraploid conditions. Chromosome size and chiasma frequency should affect multivalent frequency; it is not likely that polyploid plants with small chromosomes and few chiasmata would form multivalents as frequently as polyploids with large chromosomes and many chiasmata. The low frequency of multivalents in *Primula kewensis* (UPCOTT 1939) and *Galeopsis tetrahit* (MÜNTZING 1932) is to be noted in this connection. The hypothesis of preferential pairing of completely homologous chromosomes has been introduced (SKIRM 1942; STEBBINS 1947) to explain the meiotic regularity of natural allopolyploids. Preferential pairing demands a sufficient degree of dissimilarity between the chromosomes derived from the parental species to prevent heterogenetic association. The experiments conducted by SKIRM (1942) with the natural hybrid between *Tradescantia canaliculata* and *T. humilis* suggest that the pairing mechanism may be rather sensitive to slight differences in chromosome homology. The tetraploid produced from this hybrid by somatic doubling displayed very few multivalents at metaphase I. Presumably the pairing of exactly similar chromosomes was responsible for this. When tetraploids were obtained from unreduced gametes the proportion of multivalents was high, suggesting that the basis of preferential pairing had been eliminated in meiosis by heterogenetic exchanges between the parental genomes.

A further consequence of random chromosome association in an allopolyploid will be tetrasomic segregation of the gene differences between the parental species. Homozygosity will be rapidly approached in a diploid line subject to self-fertilization, but much more slowly in a tetraploid. Five generations of selfing on the diploid level will reduce heterozygosity to 3.1% of the original; on the tetraploid level 47% will still remain (HALDANE 1936).

The occurrence of heterogenetic chromosome association in these experiments has been established by genetic as well as cytological evidence. With 84 chromosomes, the combinational situation becomes too complex for full exploration. However, a

TABLE 7

Frequency distribution of chromosomal types in a C_2 population derived by random chromosome association in an allotetraploid of the constitution 2/2 (chromosome formula AAaa BBbb)

Genetic index	Phenotype	Chromosomal types	No.	%
8 (0)	Parental	4/4 (0/0)	2	1.0
7 (1)	Nearly parental	4/3 3/4 (1/0 0/1)	16	8.2
6 (2)	Semi-parental	4/2 2/4 (2/0 0/2)	16	8.2
6 (2)	Semi-parental	3/3 1/1	32	16.3
5 (3)	Nearly intermediate	3/2 2/3 (1/2 2/1)	80	40.8
4	Intermediate	2/2	34	17.4
4	Intermediate	3/1 1/3	16	8.2
Total			196	

simplified model will show that the decline of fertility and the correlations between fertility and morphological variation are consistent with the hypothesis of cryptic structural hybridity. Specifications for the model are as follows: the parental species each have two pairs of chromosomes; they may thus be described by the formulae AA BB and aa bb. Chromosome A is homologous with a, likewise B is homologous with b, except for a small segment reciprocally translocated between a and b. The translocated segments are interstitial rather than terminal. A gametophyte or a zygote homozygous for a deficiency is assumed to be inviable. In the allotetraploid AAaa BBbb the chief variable to be considered is the degree of preferentiality in chromosome association. Preferentiality may vary between the limits of 0 (random association) and 100% (completely homogenetic association). With random association, chromosome A is as likely to pair with a as with A. With preferentiality of 50% A will pair with A 75% of the time and with a 25% of the time. Chromosome B will behave in a similar way.

Homogenetic association will produce a single class of gametes, AaBb; but if preferentiality is incomplete eight new combinations should be produced in varying proportions. The new combinations will be AABB, AaBB, AABb, Aabb, aaBb, aabb, AAbb, and aaBB. The last two are homozygous deficient for a small segment and therefore inviable. With random association in the allotetraploid AAaaBBbb, the viable gametes will be produced in the proportion 1AABB:2AABb:2AaBB:4AaBb:2aaBb:2Aabb:1aabb. If preferentiality were acting, the frequency of the AaBb gametes would increase at the expense of the other combinations. The C_2 generation will contain 19 combinations. In table 7 the C_2 genotypes have been represented by two digits, indicating the frequency of the "A" and the "B" chromosome respectively. Thus, the hybrid AAAa BBbb is abbreviated 3/2. The "genetic index" is the sum of the numerator and the denominator of the fraction; it is a measure of the amount of material from the AABB parent. I have assumed that phenotype will be a function of the genetic index.

Of the 19 possible C_2 types, the tetraploid parental combinations will be the least frequent (1%) and the nearly intermediate classes (3/2, 2/3, etc.) the most frequent

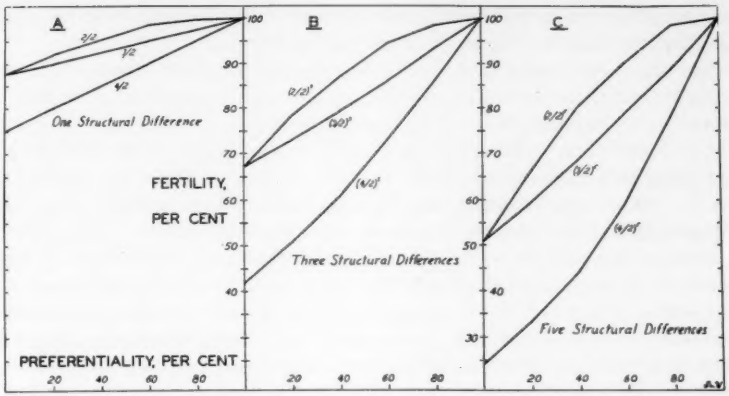

FIGURE 4. Model showing the effect of preferential chromosome association on fertility in allotetraploids with various chromosome combinations and with one, three, and five structural differences.

(41%). Fertility of each class has been computed on the assumption that fertility of the tetraploid is the frequency with which it will segregate gametes not of the constitution AAbb or aaBB. Certain C_2 classes will lack opportunity for preferential pairing; these are 3/1 (1/3), 3/3 (1/1), and 4/3 (3/4) etc. The 3/1 combination has a fertility of 75%, the others will be 100% fertile. But in the majority of the combinations (67%) fertility will be related to the degree of preferentiality in chromosome pairing. A high frequency of homogenetic pairing will result in high fertility. Furthermore, there will be a tendency for the intermediate phenotypes (those with a genetic index of 4) to have a higher fertility than those with either higher or lower genetic indices. This tendency will be particularly pronounced with an intermediate degree or preferentiality. These considerations are summarized in the "A" set of curves in figure 4. In an allopolyploid with more chromosomes than the model just discussed, the effect of a larger number of cryptic structural differences may be estimated on the assumption that they are mutually independent. Thus, if a single cryptic structural difference were responsible for a fertility of X%, then the effect of n structural differences would be a fertility of X^n%. On this line of reasoning are based the "B" and "C" sets of curves in figure 4.

Does the model explain the behavior of the tetraploid hydrids? The most striking feature of the tetraploid lines of descent is the gradual loss of fertility. In the model it is clear (table 7) that the C_1 genotype and chromosome arrangement does not reproduce itself consistently in every C_2 individual unless chromosome association is strictly homogenetic. But since the C_1 type is unstable and since the fertility of most C_2 types is less than that of the C_1, the decline of fertility with each succeeding generation of tetraploids (figure 3) seems inevitable unless selection for fertility can compensate for the instability. A further consideration is the physical basis of preferentiality, i.e. the interspecific differences between chromosomes. These differences are at a maximum in the C_1 type. Any heterogenetic association followed by crossing

over would reorganize the chromosomal material in such a way as to decrease preferentiality and hence fertility in the succeeding generations.

Another characteristic of the tetraploid population is the negative correlation between segregation for morphological characters and segregation for fertility. The properties of the model as shown in figure 4 are consistent with these observations. The C_1 constitution is phenotypically intermediate and highly fertile. Fertility in the morphologically more extreme types decreases in proportion to their resemblance to the C_1 chromosomal constitution. Linkage between factors determining hybrid morphology and those determining fertility should produce the observed correlation.

Although the evidence on this point is inconclusive, the results give some support to the theory of preferential affinity. The first point is that the number of quadrivalent associations (4 to 8 out of a possible 21) is lower than that observed in some natural or artificial autotetraploids. In *Arrenatherum elatium* (MYERS and HILL 1940), *Dactylis glomerata* (MÜNTZING 1937), *Sorghum leiocladum* (GARBER 1950) the maximum possible number of quadrivalent associations is realized in at least some of the microsporocytes at metaphase I. Secondly, taking the view that quadrivalent associations indicate random chromosome association, the differences between the several tetraploid populations are in line with the theory that preferentiality influences allopolyploid fertility. Thus the populations representing the infertile *catharticus* x *haenkeanus* hybrids have the highest frequency of multivalents (table 3). In *haenkeanus* x *stamineus* the C_2 has a lower multivalent frequency than the C_3, as well as a higher fertility. As to the degree of preferentiality, an estimate based on an average multivalent frequency of 5.0 and 7.5 per cell (table 3), assuming that all bivalents are homogenetic, gives a maximum preferentiality of 75% for the *haenkeanus* x *stamineus* hybrids and 60% in the *catharticus* x *haenkeanus* cross. Since some bivalents may be heterogenetic the actual values could be lower. A preferentiality of not more than 20 to 30% would be more consistent with the results should an estimate be based on the model described in figure 4 using the number of structural differences (table 6) and the fertility actually observed.

CONCLUSION

Association of chromosomes derived from different species (heterogenetic or illegitimate pairing) is established as a primary cause of genetic instability in these allotetraploid hybrids. The result of this instability is the segregating out of factors responsible for interspecific sterility. There is some evidence for preferential affinity; but, even if present, preferentiality is not very strong. Homogenetic association takes place not more than 20 to 30% more frequently than would be expected by chance alone.

The results were consistent with the hypothesis of cryptic structural hybridity; i.e. the theory that interspecific sterility is sometimes caused by structural differences too small to have a visible effect on chromosome pairing.

These hybrids belong to the category of segmental allopolyploids (CLAUSEN, KECK and HEISEY 1945). In plant breeding the usefulness of this category is limited by its instability which is mitigated, however, by the slow rate of segregation characteristic of the tetraploid condition, and by preferentiality. While a diploid interspecific

hybrid may be so sterile as to produce few progeny, the effect of the segregating out of sterility factors on the tetraploid level may be prolonged over several generations. Thus the use of unstable tetraploid lines of descent may be practical in the case of long-lived perennial plants.

SUMMARY

1. Diploid and tetraploid hybrids between three species of Bromus were studied to the fourth generation. All lines of descent were selected for fertility.

2. Cytological examinations of the F_1's showed perfect chromosome pairing (21 bivalents). The allotetraploids averaged 6 quadrivalent associations, almost 30 bivalents, and less than 1 univalent per cell. All the 98 cells examined at metaphase I had the expected number of chromosomes (84).

3. The diploid lines, initially highly sterile, approached full fertility by the fourth generation. The allotetraploid lines, with an original fertility of approximately 80%, decreased at a more or less constant rate to about 45% during the experiment.

4. Both the diploid and the tetraploid lines segregated for morphological characters and for fertility. Segregation of morphological characters was correlated with segregation for fertility in such a way as to suggest linkage between the fertility factors and the genes determining the morphological differences between the species.

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RELATIVE EFFECTS OF QUALITATIVELY DIFFERENT IONIZING RADIATIONS ON THE PRODUCTION OF CHROMATID ABERRATIONS IN AIR AND IN NITROGEN[1]

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DATA presented by SWANSON and SCHWARTZ (1953) confirmed and extended the findings of RILEY, GILES, and BEATTY (1952) that chromatid deletions were less markedly reduced in frequency than isochromatid deletions and chromatid exchanges when exposures were made in atmospheres of reduced oxygen tension. Since among the types of aberrations scored, chromatid deletions involve breakage only it was concluded that the reduced oxygen tension affected principally the processes of restitution. This agrees with the differential reunion hypothesis of SCHWARTZ (1952) and disagrees with the differential breakage hypothesis of GILES and his coworkers (GILES 1952; GILES and RILEY 1950; RILEY, GILES, and BEATTY 1952). This conclusion was further reinforced by the observed systematic variation with stage of cell division in the air/nitrogen ratios for isochromatid deletions and chromatid exchanges, a finding not readily explicable on the differential breakage hypothesis. Data also gave evidence that air/nitrogen ratios in these experiments were lower for chromatid and higher for isochromatid deletions than the ratios reported by RILEY, GILES, and BEATTY (1952). The only obvious difference between techniques used in the two investigations appeared to be the quality of radiation employed (RILEY et al.—250 kvp X-rays, 3 mm Al filter, HVL 0.5 mm of Cu; SWANSON—100 kvp X-rays, 1-mm Al filter, HVL ~0.04 mm of Cu). This suggested the desirability of a more systematic investigation of the oxygen effect with radiations of different quality. Such a study would be more pertinent because (1) KIRBY-SMITH and DANIELS (1953) have demonstrated the production of aberrations in Tradescantia to be dependent on the wave length of the exciting radiations, frequency of aberrations being greater the more densely ionizing the radiation, and (2) there seems to be an inverse relation between ion density produced by a given type of radiation and the magnitude of the oxygen effect on aberration production (GILES, BEATTY, and RILEY 1952; GRAY 1953a, b).

Air/nitrogen exposures, in which four different qualities of radiation were employed, produced the chromatid data in this report. Consideration of these data demanded a more precise definition of the circumstances and nature of breakage and restitution, which in turn led to the elaboration of a scheme that appears to be in keeping with the physical features of radiation as well as with the biological effects induced by these radiations.

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EXPERIMENTAL METHODS

SAX's clone #3 of *Tradescantia paludosa* was used for all experiments herein described, and all irradiations were made at room temperature (25–28°C). When inflorescences were irradiated in nitrogen, the gas was flushed through the lucite exposure chamber for 7 minutes prior to, as well as during, the exposure period; when exposed in air, the inflorescences were simply placed in the exposure chamber but without any flow of air passing over them. Acetocarmine smears were made at approximately 20 hours after irradiation.

Three qualities of X-rays, delivered by a G.E. Maxitron 250-kvp machine equipped with a beryllium window having but little inherent filtration, were employed: 50 kvp, unfiltered, HVL ~0.01 mm of Cu; 100 kvp, 1-mm Al filter (external). HVL ~0.04 mm of Cu; 250 kvp, 4 mm Cu filter (external), HVL 3.6 mm of Cu. Additional filtration was provided by 5 mm of lucite which formed the top of the exposure chamber. When only X-rays were used in comparative experiments, the milliamperage and distance were adjusted to give an intensity of 50 r per minute. When gamma radiation (1.17 to 1.33 Mev) was included with the various X-ray treatments, the X-ray intensity was dropped to 8.9 r per minute so as to be comparable to the gamma-ray intensity as measured at 30 cm from the Co^{60} source described by KIRBY-SMITH and DANIELS (1953). All measurements of dosage were made with a Victoreen ionization chamber which had been calibrated against the known intensity of the Co^{60} source.

Some question may be raised concerning the accuracy of dosage measurements made of the 50-kvp unfiltered X-rays. Although the Victoreen chamber possesses inherent limitations at the lower effective energies, much of the softer wave lengths must have been removed by the 5 mm of lucite forming the top of the exposure chamber. Any inaccuracy in the dosage measurements would probably be insufficient to invalidate conclusions to be drawn. In fact, the 50 kvp results may be omitted from consideration without disturbing the relation involved; they have been included because they show a consistency with the results from radiations of higher energy which are probably more accurately measured.

RELATION OF QUALITY OF RADIATION TO PRODUCTION OF ABERRATIONS

Table 1 presents the results of three experiments carried out at a dosage of 150 r, the quality of radiation being the important variable under consideration. Experiments A and B involve only X-ray comparisons, with the intensity maintained at 50 r per minute; experiment C includes gamma radiation as well, and was carried out at an intensity of 8.9 r per minute. Considerable variation in absolute frequencies of aberrations will be noticed among the experiments, but internal consistencies justify the assumption that these are reasonable replications of one another.

When irradiation is carried out in air, both chromatid and isochromatid deletions show a definite trend as the quality of radiation changes. As the radiation becomes harder and the ion density lessens, there is a progressive increase in the frequency of chromatid deletions and a corresponding decrease in the frequency of isochromatid deletions. It is perhaps surprising, therefore, that any increase in frequency of one type of deletion is almost exactly compensated for by a corresponding decrease in the

TABLE 1

Chromatid aberrations produced by four qualities of radiation in air and in nitrogen. Dose of 150 r in all cases

Radiation	Atmosphere	Experiment A				Experiment B				Experiment C			
		No. of cells scored	Aberrations per 100 cells			No. of cells scored	Aberrations per 100 cells			No. of cells scored	Aberrations per 100 cells		
			Chromatid deletions	Isochromatid deletions	Exchanges		Chromatid deletions	Isochromatid deletions	Exchanges		Chromatid deletions	Isochromatid deletions	Exchanges
50-kvp X-rays	Air	200	63.5	78.5	77.0	300	71.3	101.0	75.6	300	49.3	84.6	35.3
	N_2	200	102.0	15.0	16.5	300	91.6	8.6	7.8	400	98.2	15.0	9.5
	A/N_2 ratio		*0.62*	*5.2*	*2.16*		*0.78*	*11.7*	*3.1*		*0.50*	*5.6*	*1.93*
100-kvp X-rays	Air	200	77.0	64.0	62.5	300	78.3	85.6	73.3	400	66.0	62.5	30.5
	N_2	250	82.8	15.2	8.0	550	81.9	16.2	9.1	300	79.3	14.0	7.3
	A/N_2 ratio		*0.94*	*4.2*	*2.80*		*0.95*	*5.3*	*2.85*		*0.83*	*3.9*	*2.05*
250-kvp X-rays	Air	200	93.5	44.0	58.5	300	120.6	56.3	39.3	300	78.0	44.0	28.0
	N_2	200	50.5	12.5	6.5	300	72.6	13.3	4.6	300	63.3	16.6	6.0
	A/N_2 ratio		*1.85*	*3.5*	*3.0*		*1.66*	*4.2*	*2.93*		*1.23*	*2.7*	*2.16*
1.1–1.3 - Mev gamma-rays	Air									400	96.2	32.5	26.0
	N_2									400	50.5	14.0	5.0
	A/N_2 ratio										*1.9*	*2.3*	*2.28*

TABLE 2

The frequency of total deletions (chromatid plus isochromatid) and the chromatid/isochromatid ratio as a function of quality of radiation in air. Extracted from Table 1

Radiation	Experiment A		Experiment B		Experiment C	
	Total deletions	cd/iso ratio	Total deletions	cd/iso ratio	Total deletions	cd/iso ratio
50-kvp X-rays	142.0	0.81	172.3	0.71	133.9	0.58
100-kvp X-rays	141.0	1.20	163.9	0.92	128.5	1.05
250-kvp X-rays	137.5	2.12	176.3	2.14	122.0	1.78
1.1–1.3-Mev gamma-rays					128.7	2.96

other type; in other words, if the total frequency of deletions (chromatid plus isochromatid) is considered, there is no effect of radiation quality. There is a change, however, if the chromatid/isochromatid ratio is employed as a criterion of effect (see table 2).

If the assumption is made that the relations observed do not stem from some unknown but spurious experimental procedure, the only conclusion to be drawn is that the total frequency of deletions is independent of the quality of radiation employed (at least within the limits of quality used here), but that as the ion density increases a greater number of the detectable deletions are of the isochromatid type. This means that not only the effective paths of the softer radiation exhibit a progressively en-

hanced capacity to break double as opposed to single chromatids, but also, since both ineffective as well as effective ionizations contribute to dose, the softer radiation, requiring fewer ion paths to yield the same amount of ionization as the harder regions of the spectrum, exhibits a greater breakage efficiency per ion path. Such an increased efficiency is to be expected theoretically (CATCHESIDE and LEA 1943) and has been amply demonstrated experimentally by KIRBY-SMITH and DANIELS (1953). The independence of total frequency of deletions as regards quality of radiation is, on the other hand, contrary to the experimental data of KIRBY-SMITH and DANIELS. Their data, based on examination of pollen tube chromosomes of Tradescantia, show greater numbers of both chromatid and isochromatid deletions with 60- to 250-kev X-rays than with 400-kev beta or 1.17- to 1.33-Mev gamma-rays at equivalent doses, but no compensatory shift in type of deletions. Whether this difference between the two sets of data can be reconciled on the basis of the different types of chromosomes studied (i.e., pollen tube versus microspore) remains to be seen.

The chromatid exchanges produced in air show a relation to quality of radiation comparable to that exhibited by the isochromatid deletions (table 1). The differences lessen from one quality of radiation to another, but the consistency of the trend in each of the three experiments, which is in agreement with the data of KIRBY-SMITH and DANIELS (1953), suggests a real reduction in frequency as the energy of the radiation increases. The lowering of the frequency of exchanges with a lowering of intensity from 50 to 8.9 r per minute (experiment C as compared to A and B) is consistent with facts already established.

In a consideration of the frequencies of aberrations obtained from irradiation in an atmosphere of nitrogen, three features emerge. First, frequency of isochromatid deletions does not seem to be affected by the quality of radiation. The shifting frequencies obtained in the air series make these results difficult to comprehend on any basis; it may well be that the frequencies obtained in nitrogen are too low to establish a clear trend—an unlikely situation since the chromatid exchanges are even lower in frequency at the same time that a trend is strongly suggested—but the consistency of the observation for all three experiments suggests some real basis, the nature of which is not immediately clear. In any event, a shift in air without a parallel shift in nitrogen leads to air/nitrogen ratios for isochromatid deletions which are large at 50 kvp and become progressively smaller as the incident energies approach those of the gamma-rays. At 250 kvp, our ratios averaged about 3.7 and those reported by RILEY, GILES, and BEATTY (1952) averaged 2.6. The difference, however, may be attributable to the fact that the cells in our experiments were in a later stage of cell division than in theirs (SWANSON and SCHWARTZ 1953).

Second, the chromatid deletions again exhibit a relation to the quality of radiation which, in nitrogen, is exactly opposite to that found in air. This unexpected relation thus leads to actual increases in the number of chromatid deletions when 50- and 100-kvp X-rays are used and gives air/nitrogen ratios which fall below 1.0. In no instance were the air/nitrogen ratios for chromatid deletions so large as those for isochromatid deletions, although an approach to equality is obtained with gamma-rays in experiment C. The average air/nitrogen ratios for the four qualities of radiation, beginning with 50 kvp X-rays, are 0.63, 0.87, 1.4, and 1.9 respectively for chromatid deletions.

The greater frequency of chromatid deletions (aberrations which presumably involve breakage only) in cells exposed in nitrogen to medium energy X-rays than after similar exposures in air excludes the possibility that oxygen acts exclusively on the process of breakage in the manner proposed by GILES (GILES 1952; GILES and RILEY 1950; RILEY, GILES, and BEATTY 1952). In a choice between breakage and restitution as the only events which may be influenced by oxygen, the evidence supports the differential reunion hypothesis advanced by SCHWARTZ (1952) and favored by the data of BAKER and VON HALLE (1953) and SWANSON and SCHWARTZ (1953). The greater frequencies of chromatid deletions obtained by exposure of cells to 50- and 100-kvp X-rays in nitrogen as opposed to air can be visualized as arising from partial repair of breaks which, under higher oxygen tensions, would have been realized as isochromatid deletions or chromatid exchanges. This possibility was advanced earlier by BAKER and VON HALLE (1953). Whether such repair can be considered restitution, however, depends on an initial definition of breakage. This will be elucidated in the discussion.

Third, the chromatid exchange air/nitrogen ratios, calculated on the basis of $\sqrt{\text{frequency in air}}/\sqrt{\text{frequency in nitrogen}}$ since exchanges vary approximately as the square of the dose, show a relation to quality of radiation similar in trend to that for chromatid deletions. This trend is expected on the likely assumption that chromatid exchanges arise from the illegitimate reunion of breaks similar to chromatid deletions whereas the reduction in exchanges resulting from irradiation in nitrogen can be explained by the removal, through partial restitution, of those chromatid breaks which would normally have contributed to the formation of exchanges.

DOSAGE RELATIONS

Two predictions can be made from the data in table 1. First, on the basis of the linear relation between frequency of chromatid deletions and dose, the curve for these aberrations induced by 50-kvp X-rays in nitrogen would be expected to be steeper than that for irradiation in air, whereas the reverse situation should hold for 250-kvp X-rays. These expectations are realized (see fig. 1) and the air/nitrogen ratios in this instance were approximately 0.66 and 1.54, respectively, for the curves as a whole. The data in figures 1 and 2 were obtained in the present experiments from irradiation at 50 r per minute, under the conditions described. Not less than 200 cells were observed for each dose point.

Second, a prediction can be made that irradiation in nitrogen will effect a change in the shape of the isochromatid curve. KIRBY-SMITH and DANIELS (1953), following an earlier study of CATCHESIDE, LEA, and THODAY (1946), have demonstrated conclusively a departure from linearity of the isochromatid curve which is caused by a proportion of the aberrations resulting from two independent hits, this 2-hit component being a larger fraction of the total, the higher the energy of the radiation. Therefore, when a particular type of aberration such as an isochromatid deletion can arise through either a 1- or 2-hit event, it would be expected that any factor favoring a reduction in total frequency through restitution would preferentially reduce the 2-hit as compared to the 1-hit type. Such a deduction is logical on the basis of the time element involved in the production of 2-hit isochromatid deletions, and the result would be not only a reduction in total frequency but also a change in

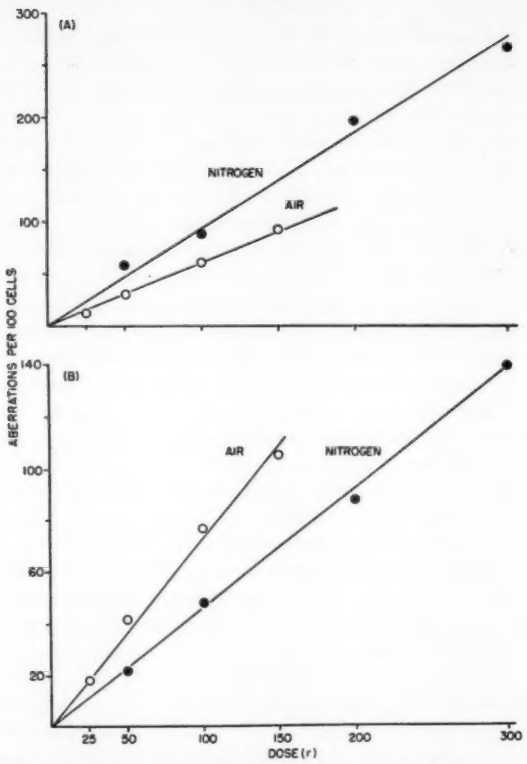

FIGURE 1. Relation of chromatid deletions to dose in air and in nitrogen at two qualities of radiation. (A) 50-kvp X-rays unfiltered; (B) 250-kvp X-rays filtered through 4 mm of Cu. The vertical scale of (B) is 2½ times that of (A).

the shape of the dosage curve from a nonlinear character to one more nearly approaching linearity. The data obtained to test this hypothesis are given in figure 2. The curves for irradiation in air have powers of 1.51 and 1.67 for the 50- and 250-kvp X-rays respectively. These are in numerical agreement with a similar curve obtained by THODAY (1953) in Vicia, but somewhat higher in value than the powers previously reported by KIRBY-SMITH and DANIELS (1953).

In nitrogen, the corresponding curves have values of 1.08 and 1.27, and the respective P values are 0.019 and 0.036. This, of course, leads to increasingly larger air/nitrogen ratios as the dose increases. Although certain misgivings may be attached to the high values obtained in air, the data are in accord with the stated hypothesis. Support is also provided by the isochromatid data of RILEY, GILES, and BEATTY (1952). A re-analysis of their curves with 250-kvp X-rays (3 mm of Al filtration) yields values of 1.22 and 1.04 for air and nitrogen, respectively, with the air/nitrogen ratios varying from 1.46 at 50 r to 2.66 at 150 r.

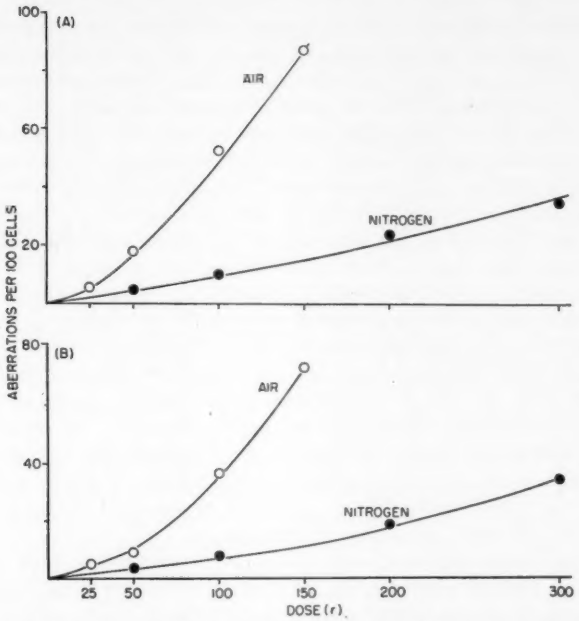

FIGURE 2. Relation of isochromatid deletions to dose in air and in nitrogen for two qualities of radiation (A) 50-kvp X-rays unfiltered; (B) 250-kvp X-rays filtered through 4 mm of Cu. Air/nitrogen ratios at 50 kvp are 3.7 at 50 r, 4.7 at 100 r, and 5.8 at 150 r; at 250 kvp the ratios at the same doses are 2.4, 4.6, and 5.7, respectively.

The chromatid exchanges produced by radiations in the X- and gamma-ray regions of the spectrum are already known to be 2-hit aberrations, the power of the dose curve approaching 2 at high radiation intensities. There is no *a priori* reason for assuming that the atmosphere maintained during irradiation has any influence on the shape of the curve even though the frequency is reduced. RILEY, GILES, and BEATTY (1952) have demonstrated the truth of this for chromosomal exchanges, and evidence obtained during these dose studies on chromatid exchanges confirms it. The air/nitrogen ratios, however, are slightly under 4.0 for the 50-kvp X-rays as compared to about 2.8 for the 250-kvp radiation, thus agreeing with the data on isochromatid deletions which point to the greater ratios obtained with softer radiations for aberrations involving the illegitimate union of broken ends.

DISCUSSION

Interpretations of the oxygen effect on irradiated chromosomes have been based largely on the initial assumption that one or the other of two processes is oxygen dependent. These are breakage and restitution, and up to the present time proponents of the two opposing points of view have considered the available data in rather unequivocal terms. However, an attempt to fit the present findings, along with previous

X-ray, neutron, and alpha-ray data, into a meaningful hypothesis, produces evidence that neither the differential breakage hypothesis of GILES nor the differential reunion hypothesis of SCHWARTZ is entirely satisfactory without further elaboration. Operationally, breakage and restitution are quite different and separable processes, but if one considers, as THODAY (1953) has done, that latent as well as actual breaks are induced by radiation and that oxygen may exert its influence by determining the number and/or the fate of the latent breaks, then more refined definitions become necessary. If, as commonly used, "breakage" means the physical rupture of chromatin strands, and "restitution" the restoration of the original structural continuity of the chromosome, and, further, if we consider these two processes to be the only relevant parameters subject to possible modification by oxygen tension, then the bulk of the data forming the present report supports the hypothesis that the restitutive processes are oxygen dependent whereas breakage is a function solely of dose and quality of radiation. The greater frequency of chromatid deletions obtained in nitrogen as compared to air with 50- and 100-kvp X-rays (table 1) cannot be explained by assuming that breakage alone (as defined here) is affected by the oxygen tension in the cells. Restitution of some sort must be involved, and it is believed that the differential air/nitrogen ratios obtained for chromatid aberrations are a reflection of a process of "partial restitution" which transforms potential isochromatid deletions and chromatid exchanges into chromatid deletions.

It should be emphasized that the differential air/nitrogen ratios of the sort found among chromatid aberrations would not be expected to hold for aberrations induced in chromosomes which are single-stranded at the time of irradiation. Restitution, whatever its nature, would simply restore a broken chromosome to its former intact self, and thus reduce the frequency of all types of aberrations equally.

Let it be pointed out at once, however, that this scheme does not provide unqualified support for the contention that restitution, as defined here, is oxygen dependent. In fact, the neutron and alpha-ray data strongly suggest otherwise, and a consideration of the data in table 2 will make this clear. As the ion density of the radiation increases, the chromatid/isochromatid ratio decreases. With the more densely ionizing particulate radiations, the chromatid/isochromatid ratios are likely to be smaller than those resulting from X- and gamma-ray exposures; it would be expected, therefore, that the air/nitrogen ratios for chromatid deletions would be smaller, and for isochromatid deletions larger, than those obtained from exposure to 50-kvp X-rays. That they are not has been clearly demonstrated by THODAY and READ (1949) for alpha-rays and by GILES, BEATTY, and RILEY (1952) for neutrons.

A recalculation of the data in table 1 in terms of a percentage reduction in total breakage as the result of irradiation in the absence of oxygen reveals, however, a definite relation of oxygen effect to quality of radiation (table 3). As already pointed out by GILES, BEATTY, and RILEY (1952), and as re-emphasized by GRAY (1953a, b), the relation is inverse in that the greater the ion density of the radiation the less is the effect of oxygen tension in determining the frequency of aberrations. In table 3, the figures in experiment B do not agree with the other pieces of data, but it is believed that this departure results largely from a marked discrepancy in the 50-kvp category (table 1) as indicated by the very large air/nitrogen ratios for isochromatid

TABLE 3

Percentage reduction in total breakage as the result of exposure in the absence of oxygen. Total breakage calculated by multiplying chromatid deletions and isochromatid deletions by 1, and chromatid exchanges by 2, and adding them together

Radiation	Expt. A	Expt. B	Expt. C	Miscellaneous*
1.1–1.3-Mev gamma-rays			59	
250-kvp X-rays	70	63	48	58
100-kvp X-rays	57	63	43	
50-kvp X-rays	43	65	36	
Neutrons				33
Alpha-rays				0

* Note: Reduction of 58% at 250-kvp radiation was extracted from RILEY, GILES, and BEATTY (1952; table 1 at 150 r). The neutron and alpha reductions were derived from data in GILES, BEATTY, and RILEY (1952; table 2 at 10 n). THODAY and READ (1949) indicate that some reduction is obtained with alpha-rays in nitrogen.

deletions and exchanges. Consequently, it must be concluded that the neutron and alpha-ray data, when considered together with the data in table 1, are in conflict with the idea that oxygen tension is a limiting factor in governing the degree of restitution as herein defined, even as the increases in chromatid deletions in nitrogen as opposed to air at 50- and 100-kvp X-rays cast suspicion on the validity of the differential breakage hypothesis.

How then is "partial restitution" to be defined and at what level of events can it take place? For purposes of definition, partial restitution is considered to be a process by which potential isochromatid deletions and chromatid exchanges are converted into actual chromatid deletions. Both isochromatid deletions and chromatid exchanges involve two broken chromatids, followed by illegitimate reunions (with the exception of the NUpd type of isochromatid deletion); restitution of one of the broken chromatids would therefore convert potential isochromatid deletions and exchanges into chromatid deletions. Furthermore, it is considered that partial restitution involves latent rather than actual breaks, and that the latter are unaffected by oxygen once they are formed. Although this concept will be more fully developed in a later report, it may be outlined briefly as follows: the immediate effects of radiation involve the production of both actual and latent breaks, the latter differing from the former by a delay in the separation of broken ends. Both THODAY (1953) and GRAY (1953b) give excellent summaries which support the concept of the latent break being produced by ionizing radiations. The total frequency of breaks, both actual and latent, is oxygen independent, but the relative frequencies of the two types, which are likely to be simply the termini of a continuous spectrum of breaks rather than categories distinct from each other, will be independent of dose but dependent on the ion density of the radiation, i.e., the actual breaks will be a relatively larger proportion of the total break frequency, the greater the ion density. Ion density will further determine the relative frequencies of single chromatid versus isolocus chromatid breaks, whether latent or actual. In this situation, and on the assumption that partial restitution is concerned with the fate of latent breaks to the extent that anoxia

favors their removal while the presence of oxygen favors their transformation into actual breaks, the relation of oxygen effect to quality of radiation as well as the differential air/nitrogen ratios for chromatid aberrations can be explained. The differences in yield of aberrations caused by irradiation in nitrogen as compared to air reflect, therefore, a difference in the frequency of actual breaks.

It is not to be supposed that the hypothesis presented adequately encompasses all aspects of the oxygen effect on irradiated chromosomes. In fact, the fractionation experiments of WOLFF and ATWOOD (1954) indicate that the effects of X-rays on breakage and reunion are independent of each other, while studies of X-ray-induced stickiness of chromosomes in air and in nitrogen suggest interrelations which have not yet been resolved (SWANSON and JOHNSTON, in press). The data presented in this report cannot, because of their nature, be appraised as regards these points.

SUMMARY

A comparative study of frequencies, in air and in nitrogen, of chromatid aberrations caused by 50-, 100-, and 250-kvp X-rays, as well as by Co^{60} gamma radiation has shown the following:

1. In air, chromatid deletions increase and isochromatid deletions decrease in frequency as the radiation becomes more energetic, but the compensatory shift in ratio between the two types is such that the total frequency of deletions reveals no wave-length dependence.

2. In nitrogen, the relation of chromatid deletions is the opposite of that in air, and with 50- and 100-kvp X-rays, the frequencies are higher than in air. The air/nitrogen ratios for chromatid deletions, therefore, are below 1.0 with the 50- and 100-kvp X-rays, and above 1.0 for 250-kvp X- and gamma-rays.

3. In nitrogen, isochromatid deletions show no dependence on quality of radiation. The resultant air/nitrogen ratios are highest with 50-kvp X-rays, and show a steady decrease in value as the radiation energy increases.

4. Chromatid exchanges show a slight, but entirely consistent, relation to quality of radiation, and the air/nitrogen ratios follow a trend similar to that found for chromatid deletions.

5. The power of the isochromatid dose curve is reduced in value when exposures are carried out in nitrogen, due largely, it is believed, to a preferential reduction in the 2-hit type of isochromatid deletion.

6. On the basis of total breakage, the oxygen effect is more pronounced the more energetic the ionizing radiation.

If it is considered that breakage and restitution are the only possible events subject to an oxygen dependence, the data presented are meaningful only in terms of the differential reunion hypothesis of SCHWARTZ (1952). Reasons are given for the belief that the existing hypotheses are too narrowly conceived to provide for all of the data available. An attempt has therefore been made to incorporate existing data into a modification of THODAY'S scheme (1953), a modification which considers the latent break to be a reality, and which postulates that the major effect of oxygen is such as to determine the fate of latent breaks.

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A GENETIC ANALYSIS OF SECTORING IN ULTRAVIOLET-INDUCED VARIANT COLONIES OF YEAST

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A VARIATION from galactose positive to galactose negative has been found in *Saccharomyces cerevisiae* that is exceptional in that it can be induced with very high frequency by ultraviolet irradiation (JAMES 1954). The variant lines often occur as sectors in otherwise phenotypically normal colonies grown from irradiated vegetative cells on galactose-EMB indicator plates. The nature of the induced change has been studied by determining the genotypes of (a) the parental strain, (b) isolates from negative (variant) sectors, (c) the associated positive sectors, and (d) phenotypically unaffected colonies occurring in the same plates.

Previous breeding tests indicated that the parent line is heterozygous for the galactose character (positive being dominant), and that the induced variant is homozygous recessive. These facts suggested that the variant arose through mutation of the dominant gene in the heterozygote (see also WINGE and ROBERTS 1950). The sectoring, which there is reason to believe is more frequent than would appear from gross examination of the colonies (JAMES 1954), could be the result of delayed mutation. It would be possible, however, to account for both the high frequency of the change and the sectoring on the hypothesis that radiation induces genetic segregations in vegetative cells. Evidence for this is presented in the following account.

MATERIAL AND METHODS

Strain 562 of *Saccharomyces cerevisiae*, used in these investigations, is a descendant of CRI which has been described elsewhere (JAMES 1954). This strain shows a lower induced variant frequency than CRI, but has been used because of the low frequency of ascospore germination in the latter. The pedigree of strain 562 will be given later in a discussion of its genetic constitution.

The galactose-EMB indicator medium and the method of irradiation were similar to those used previously in this laboratory (JAMES 1954). An ultraviolet dose of 200 ergs/mm^2 was used in all irradiations.

Determinations of genetic constitutions of variant and non-variant colonies were made with clones derived by picking, dilution and replating, followed by single colony isolation. Sex was determined by incubating with two tester stocks of opposite sex. Cultures were also incubated alone to detect any anomalous behaviour. Galactose reaction was determined by plating 50 to 200 cells on indicator medium and observing the resultant colonies.

Two mating systems were used. A mass mating technique with isolation of apparent diploids (FOWELL 1951) was used in the series of crosses leading to strain 562. All other crosses were made with the technique of mating individual cells and isolating resultant zygotes (CHEN 1950).

RESULTS

Variant cells are induced in strain 562 with an estimated frequency of 1.7 percent among cells surviving an ultraviolet dose of 200 ergs/mm^2. With this dose the proportion of variant colonies that are sectored is approximately 67 percent. A vast majority of the variant cells are stable on transfer.

The investigation consisted primarily of a comparison of the genetic constitutions of 12 sectored colonies and 12 phenotypically normal colonies produced by irradiated cells. Each sectored colony was represented by a positive (b) clone and a negative (w) clone, and each wholly positive colony by a single positive clone. The genetic constitutions of these were determined in regard to galactose, sex and lethal genes. The results obtained are summarized in table 1.

The clones were found to have a variety of aberrant genotypes; in addition to those 12 negative clones that were homozygous recessive for galactose, 11 of the 36 were heterozygous for recessive lethals, 3 were homozygous for sex, 9 were homozygous for dominant galactose genes, and one was tetraploid. Further, the positive clones from sectored colonies showed a greater number of aberrations for the galactose character than did positive clones derived from phenotypically normal colonies; seven of the clones from positive portions of sectored colonies were homozygous dominant, whereas only two of the clones from phenotypically normal colonies were aberrant. The correlation in appearance of aberrant positive and negative variant cells suggests that genetic exchange, rather than gene mutation, is the mechanism producing the variant cells. In subsequent sections this possibility is examined critically.

Heterozygous origin of sectored colonies

The classification of strain 562 as a diploid heterozygous for a single gene pair controlling the galactose character was based on its ancestry and on the results of progeny tests. The strain was obtained from a negative segregant (385) of a tenth generation inbred line of CRI. No. 385 was crossed to the positive haploid ATCC 10275. This mating was followed by first and second generation backcrosses of negative segregants to ATCC 10275, the latter resulting in strain 562. Each zygotic generation was galactose positive, and heterozygosity throughout the pedigree was inferred when segregants from three asci of the F_1 generation and from three asci of the first generation backcross produced regular 2:2 ratios for galactose and for sex.

In a progeny test of strain 562, the four segregants from each of 20 asci were tested for galactose reaction, and mating reaction was determined in the segregants from 14 of the same asci. All the segregations were completely regular.

Genetic analyses of isolates

Of the 36 clones representing the twelve sectored and twelve phenotypically normal colonies in table 1, three (2w, 5w and 18) failed to sporulate and one (2b) produced irregular segregation ratios. The analyses of these four clones will be considered later. The analyses of the remaining 32 clones were made primarily with data obtained from ascus dissections, and they will be considered as a unit. Segregation data for sex and galactose genes were obtained only from those asci that produced four spore colonies unless the clones contained recessive lethals In these cases, only two spore

TABLE 1

*Genetic constitutions of isolates from twelve sectored and twelve phenotypically normal colonies formed by irradiated vegetative cells of genotype $AaLLL_1L_1Gg$**

(Dose: 200 ergs per mm.^{2})

Genetic constitution†		No. of colonies observed	Colony number (in order of picking)
Positive sector	Negative sector		
Sectored colonies			
Aa LL Gg	*Aa LL gg*	2	9, 11
Aa Ll Gg	*Aa Ll gg*	2	1, 6
Aa LL GG	*Aa LL gg*	5	4, 7, 8, 10, 12
Aa Ll GG	*Aa Ll gg*	1	3
Aa Ll L_1L_1 GG	*aa L- L_1- gg*	1	5
AA aa LL LL GG gg	*aa L- gg*	1	2
Positive (phenotypically wild type) colonies			
Aa LL Gg	—	6	13, 15, 17, 20, 23, 24
Aa Ll Gg	—	4	14, 16, 19, 22
AA L- GG	—	1	18
Aa LL GG	—	1	21

* *A*, *a*:—sex, *L*, *l* and L_1 l_1:—lethals, *G*, *g*:—galactose.

† The genetic constitutions are from tests of a single isolate from each sector or colony and may or may not apply to the whole of the sector (or colony).

colonies per ascus could be examined. Ten asci were studied from each of the positive clones, with the exception of clone 20 which sporulated so poorly that only two complete asci could be obtained from it. Negative clones were represented by two asci if lethals were absent or by four if lethals were present.

Eleven of the 32 clones were classified in table 1 as having originated from isolates containing recessive lethals. This classification was based on the frequencies with which dissected spores produced colonies; in a total of 239 asci, not fewer than five from any one clone, none produced more than two viable spore colonies. On the other hand, 76 percent of 363 asci from clones designated free of recessive lethals produced either three or four spore colonies. One clone, 5b, was classified as heterozygous for two recessive lethals because the distribution of asci producing 0, 1, and 2 spore colonies was 19, 30, and 11.

Data on the segregation of sex genes were in close accord with the proposition that all 32 clones were normal diploids. Of the 146 tetrad analyses, 125 were completely orthodox. Of the 92 diad analyses, 84 were apparently orthodox. Only one type of deviation from regularity occurred. This was displayed by those spore cultures that exhibited a mating reaction with both tester strains. This occurred in 43 of the 768 spore cultures tested. In 40 of these a mating reaction was also observed in the unmated control cultures. Such anomalous behaviour has been attributed to spontaneous mutations for sex prior to testing (AHMAD 1952; POMPER and MCKEE 1953). Consistent haploidy of the segregants was further indicated when attempts to sporulate 428 of the spore cultures were unsuccessful in all cases.

TABLE 2

Details of segregation of galactose character in isolates of colonies formed by irradiated cells

	Genotype	No. of colonies	Type of analysis	Ratio pos.:neg. spores in ascus	No. of asci	Colony number
Sectored colonies						
Negative sectors	*gg*	7	tetrad	0:4	14	4, 7, 8, 9, 10, 11, 12
		3	diad	0:2	12	1, 3, 6
Positive sectors	*Gg*	2	tetrad	2:2	20	9, 11
		2	diad	2:0	4	1, 6
				1:1	13	
				0:2	3	
	GG	5	tetrad	4:0	50	4, 7, 8, 10, 12
		2	diad	2:0	20	3, 5
Phenotypically normal colonies	*Gg*	6	tetrad	2:2	52	13, 15, 17, 20, 23, 24
		4	diad	2:0	13	14, 16, 19, 22
				1:1	22	
				0:2	5	
	GG	1	tetrad	4:0	10	21

Scoring of the galactose character was unequivocal on the indicator medium. There was no evidence of heterogeneity in galactose reaction among the colonies of negative segregants, and among positive segregants heterogeneity was limited to three spore cultures. Even in these three instances the heterogeneity was slight, consisting of a maximum of two negative colonies among the several hundred positive colonies on test plates.

The galactose segregation data summarized in table 2 indicate that a single locus is concerned in the galactose reaction. The ten negative clones gave no indication of being other than homozygous recessive, since all 80 segregants from 26 asci were negative. Further evidence for the homozygous recessive condition of negative clones was obtained when the four negative spore cultures of an ascus of 4w were mated to positive haploids (progeny of strain 562, unirradiated). All the resultant cultures were positive.

It seems unlikely that the negative character is due to the complete loss of a locus in view of the existence of reversions which can occur, if not at the original locus, then close to it. Such reversions appear as papillae on negative colonies. Eleven reversions of independent origin segregated as heterozygotes. One of the positive segregants from a reversion of 7w was mated to a positive segregant of strain 562 (unirradiated). None of the spore cultures from eleven segregations of the resultant diploids (four from one mating, seven from the other) was negative.

The 22 positive clones fell into either of two distinct classes with respect to their segregations for galactose, one indicating heterozygosity (*Gg*), the other indicating homozygosity (*GG*). That any of the clones in this latter class were dihybrids for galactose seems most unlikely in view of the complete absence of negative spore cultures among 280 segregants.

Confirmation of the genetic difference between those clones classified as heterozygous and those classified as homozygous dominant was found in a comparison of in-

TABLE 3

Induced variant frequencies of cultures of different genotype

(Dose: 200 ergs/mm²)

Genotype	No. clones	No. irradiations	Colonies scored	Percent induced variants*
Gg	14	56	98,431	1.8
GG	8	32	74,326	0.2
G	5	17	42,178	0.1
gg	4	8	15,550	0.03

* Combined data. Percent variants by individual clones:

Gg:—1.7, 1.6, 1.9, 1.3, 1.9, 2.4, 1.7, 1.7, 1.6, 2.2, 3.4, 1.6, 2.2, 2.0

GG:—0.1, 0.4, 0.3, 0.2, 1.0, 0.2, 0.1, 0.2

G:—0.4, 0.2, 0.1, 0.1, 0.04

gg:—0.00, 0.04, 0.04, 0.02

duced variant frequencies (see table 3). The 14 assumed heterozygotes have induced frequencies similar to that of strain 562. The frequencies of the eight assumed homozygotes ranged between 0.1 and 0.4 percent with the exception of clone 3b which had an average induced variant frequency of 1.0 percent. This high frequency is unexplained although it might be attributed to the spontaneous appearance of heterozygous cells within the clone.

The actual difference between the two genotypes in induced variant frequency is undoubtedly greater than the data imply. These data are uncorrected for those colonies that were scored as variants but which were not stable on transfer. Such colonies, when replated, produce either typically positive or brownish colonies. Although the number of such colonies is low, in those cultures where the total variant count is also low their contribution to the estimated variant frequency is large. In later irradiations of homozygous diploids all colonies scored as variant were tested by replating. In these tests, of 16 variants scored among 13,120 colonies of 4b, 12b and 21, only two were stable, indicating a corrected frequency of 0.02 percent.

Data concerning the linkage relationship of sex and galactose are shown in table 4. There is no indication of linkage, the numbers of the two ditypes being 27 and 22. Linkage of lethals with sex and with galactose genes would be indicated for individual clones by inequality of 2:0 and 0:2 diad segregations. No significant indication of linkage with galactose was found. Linkage with sex was indicated in one clone (19) where the ratio of 2:0 to 0:2 segregations was 7 to 0 (significant at 1 percent level).

Clone 2b produced segregation ratios typical of a tetraploid heterozygous for galactose. Data were obtained from ten asci. Of the segregations for the galactose character, seven were 4:0, one was 3:1, and two were 2:2, positive to negative. Irregularities in the segregation of sex genes were demonstrated by the fact that 16 of the 40 spore cultures failed to give any mating reaction. Because of this, all 40 spore cultures were tested for ability to sporulate. With one exception, positive mating reactions were accompanied by failure to sporulate, whereas negative mating reactions were coincident with an ability to sporulate. These results are consistent with the supposition that 2b was tetraploid, since non-mating spore cultures with ability

TABLE 4

Absence of linkage between sex and galactose

Clone	No. of asci tested	Distribution of ascus types		
		Parental ditype (*aG, aG, Ag, Ag*)	Non-parental ditype (*AG, AG, ag, ag*)	Tetratype (*AG, Ag, aG, ag*)
Parental strain	14	3	5	6
Sectored colonies				
9b	10	1	3	6
11b	10	3	3	4
Phenotypically normal colonies				
13	10	6	2	2
15	10	4	2	4
17	10	4	2	4
20	1	0	1	0
23	10	4	1	5
24	10	2	3	5
Total	85	27	22	36

to sporulate are suggestive of heterozygosity for sex, and segregants with ability to mate could be homozygous for sex. The four non-mating segregants of one ascus were sporulated. Two asci from each segregant, when tested for galactose reaction, were found to have segregated 2:2, confirming the conclusion that 2b was a tetraploid heterozygous for galactose and demonstrating that it was free of recessive lethals.

The genetic analyses of the two negative clones 2w and 5w and of the positive clone 18 were less complete than those of the other clones because they could not be induced to sporulate. On testing for sex, 2w and 5w gave reactions indicative of sex "a" and 18 gave a reaction indicative of sex "A". In an attempt to determine the ploidy of these clones, they were mated to haploid segregants of strain 562 (unirradiated) of opposite galactose reaction. The three resulting zygotic cultures were galactose positive and sporulated abundantly. Ten asci from each mating were dissected. The matings of 5w and 18 produced no viable segregants; the mating of 2w produced 2, 1, and 0 spore colonies per ascus with frequencies 3, 1, and 6. On the basis of these results, indicating triploidy, the three original clones were classified as diploids homozygous for sex. The classification of clone 18 as homozygous for galactose was based on its induced variant frequency (0.2 percent of 9,310 colonies) which was closely comparable to the low frequency of clones known to be of that genotype.

The uninuclear origin of sectored colonies

Evidence that the sectored colonies under study were derived from single irradiated nuclei was obtained from the distribution of lethals within these colonies.

That the majority of these lethals were of irradiation-induced origin was demonstrated in a test utilizing a single culture of 9b. Twenty clones from positive portions of sectored colonies and 20 clones from phenotypically normal colonies were obtained from platings of irradiated cells. Twenty control clones were also obtained from platings of non-irradiated cells. These 60 clones were sporulated and spore germination

tests were made. A clone was designated free of recessive lethals if any dissected ascus produced three or four spore colonies, but was designated heterozygous for a recessive lethal if not more than two spore colonies could be obtained from any ascus with a minimum of eight dissections. No lethals were found in the controls, seven lethals were found in the 20 clones from sectored colonies, and seven lethals were found in 19 of the clones from normal colonies, one clone failing to sporulate. It is apparent that these lethals were irradiation-induced. And since the proportion of colonies which contained lethals was almost identical to that of the previous test (36 percent as compared to 35 percent in table 1) there can be little doubt that the lethals of that test were also irradiation-induced.

With this information, the distribution of lethals within sectored colonies (see table 1) provides good evidence that the majority of these sectored colonies were derived from single irradiated nuclei. Of the ten sectored colonies (omitting colonies 2 and 5 which could not be completely analyzed), presence or absence of a lethal in the positive portion was consistently accompanied by a corresponding presence or absence in the negative portion. If all these colonies had been formed by the seeding of two nuclei at one locality, the probability of obtaining such a distribution is less than one in 1000.

Correlated induction of homozygous dominant and homozygous recessive genotypes

The analyses of colonies from irradiated cells of strain 562 showed that more sectored colonies than phenotypically normal colonies contained homozygous dominant cells. However, the proportions (7/12 and 2/12) do not differ significantly. It was consequently considered necessary to determine whether the observed difference in proportions could be attributed to sampling error.

This was accomplished by determining the genotypes in regard to galactose, of the 60 isolates from 9b that had already been analysed for lethal genes. The isolates were tested only to provide evidence of homozygosity or heterozygosity, and the validity of the proposed genotypes was based wholly on the consistency of the segregations in more thoroughly analyzed clones. With those clones that did not contain a recessive lethal, tests were confined to segregants (either three or four) from a single ascus. With those clones that contained a lethal, paired segregants were tested. If no negative spore cultures appeared among the paired segregants of four asci, the clone concerned was classified as homozygous. If fewer than four paired segregants were available and heterozygosity remained in doubt, genotype was inferred from induced variant frequency. Ten homozygous dominant clones were found among the 20 representatives of sectored colonies, whereas none was found among the 20 representatives of control platings or among the 20 normal colonies from platings of irradiated cells. Although the tests contained no analysis for the presence of occasional tetraploids, there can be little doubt that the occurrence of homozygous dominant cells within colonies is correlated with that of homozygous recessive cells. The combined results of the two sets of analyses (involving strain 562 and clone 9b) indicate that 17 of 32 clones from positive sectors were homozygous dominant while only two of 32 clones from phenotypically normal colonies were homozygous dominant. The difference is highly significant.

Induced variant frequency of positive haploids and homozygous recessive diploids

The correlated appearance of homozygous dominant and homozygous recessive cells prompted a determination of the induced variant frequencies of positive haploids, and homozygous recessive diploids. In the case of positive haploids, ATCC 10275 (the source of the positive character in strain 562) and four segregants from an ascus of 7b were irradiated and plated. The results are included in table 3. The variant frequencies of the haploids are significantly lower than those of heterozygotes, averaging 0.1 percent. All colonies scored as variant were picked and replated. Of the 38 colonies so tested, 23 proved to be stable, indicating a corrected frequency of 0.05 percent.

In tests of the induced variant frequencies of homozygous recessive diploids, cultures of 4w, 7w, 9w, and 12w were irradiated as above. Counts were made of induced reversions among the resultant colonies. The results of these tests (see table 3) indicate that the frequency of induced reversions in homozygous recessive diploids is much lower than the frequency of homozygous dominants which appear after irradiation of heterozygotes.

DISCUSSION

The present study indicates that genetic segregations occur in yeast as a result of ultraviolet irradiation of diploid vegetative cells. It should be noted that both spontaneous and irradiation-induced segregations have been observed in the somatic cells of Drosophila (STERN 1936; WHITTINGHILL 1950), and that spontaneous segregations also occur in Penicillium and Aspergillus (PONTECORVO *et al.* 1954). Segregations, both spontaneous and irradiation induced, that may be somatic, have also been observed in exceptional heterozygotes of *E. coli* (LEDERBERG 1949; LEDERBERG *et al.* 1951).

In the present material, not only does gene reassortment supply a simple explanation of both the high frequency of variants and the high frequency of sectoring, but it also accounts for the correlated appearance of homozygous dominant and homozygous recessive cells. Further, the occurrence of a high induced frequency of homozygous recessive and homozygous dominant variants in heterozygous diploids, a low induced frequency of negative variants in positive haploids, and a low induced frequency of positive variants in homozygous recessive diploids is to be expected on such a hypothesis.

The occurrence of wholly negative colonies, and of wholly positive colonies that contain homozygous dominant cells (18 and 21), is not necessarily contrary to a hypothesis of gene segregation since the presence of such colonies can be attributed to elimination of some lines of descent from irradiated cells (JAMES 1954). Similarly, the failure to isolate complementary homozygous dominant genotypes from some sectored colonies is not critical since analyses were confined to single isolations from each sector and it may well be that some positive sectors contained a mixture of homozygous and heterozygous cells. The presence of heterozygous cells in sectored colonies does, however, imply that such exchanges sometimes occur subsequent to at least one normal mitotic division.

Although these studies have not excluded gene mutation, they have supplied information that is difficult to interpret on such a basis. Explanations for the corre-

lated appearance of homozygous dominant and homozygous recessive cells might be found in the assumptions that (1) delayed dominant mutations actually occur independently of negative variants but that selection pressures tend to eliminate them in colonies that do not contain homozygous recessive cells, or that (2) an extreme heterogeneity in sensitivity to irradiation exists between cells. The first assumption demands that dominant mutations be induced with a frequency of at least 50 percent. Evidence unfavourable to the second assumption is found in that data on induced recessive lethals. These were distributed independently of sectoring; 11 of 31 sectored colonies and 11 of 31 phenotypically normal colonies contained such lethals. Further, to explain the correlation of induced variant frequency with the heterozygous condition of cells, it would be necessary to assume either that mutation is dependent on the presence of an allele identical to the mutant allele, or that reproduction of the mutant cell is dependent on the presence of such an allele. Gene segregation, however, seems to involve less complicated assumptions.

There are several means by which reassortment could be induced. At this time the most likely of these appears to be (1) somatic crossing over or (2) disjunction of homologous chromosomes. Reciprocal chromatid translocation seems unlikely as a mechanism because those derived from random chromatid breaks would be expected to produce a heterozygous lethal condition in one sector of the colony, whereas such lethals as were found affected both sectors.

Induced meiosis also appears to be an unlikely mechanism. (1) If meiosis contributes to variant production one would expect clones of phenotype *AAGg* or *aaGg* to exhibit a low induced variant frequency since these do not ordinarily undergo sporulation. Tests with two such clones have shown that this is not the case; both exhibited variant frequencies similar to that shown by clones of genotype *AaGg*. (2) Although aberrations in cell morphology are frequent following irradiation, nothing resembling an ascus has been noted. (3) If meiosis were a factor, one might expect the occurrence of many haploid clones, and these have not been found. (4) It seems unlikely that each of the three clones, 2w, 5w, and 18, which were homozygous for sex, resulted from the fusion of haploids.

At this time it seems likely that irradiation-induced variations of high frequency are not peculiar to the locus under investigation here or to the particular strain of yeast. This is suggested by the high frequency of maltose variants in hybrids of *S. cerevisiae* and *S. Chevalieri* (WINGE and ROBERTS 1950). Also, we have noted a high frequency of induced galactose negative variants (greater than 2 per cent) in a strain of *S. cerevisiae* unrelated to that used in these studies.

SUMMARY

Evidence that irradiation of vegetative diploid cells of yeast induces a reassortment of genetic loci has been obtained using a galactose positive strain of *Saccharomyces cerevisiae*. Variant negative colonies are produced in this strain with a high frequency approaching 2 percent of survivors when cells are irradiated with an ultraviolet dose of 200 ergs/mm^2 previous to plating. With this dose, approximately 67 percent of these are sectored, containing both phenotypically normal and variant cells. Investigations were centered on a genetic analysis of sectored colonies.

Results have indicated that (1) parental cells are heterozygous for a single gene pair controlling the galactose character, (2) variant negative cells are homozygous recessive, (3) sectored colonies are derived from single irradiated nuclei, (4) irradiation of heterozygotes induces homozygosity for dominant genes as well as for recessive genes, (5) the appearance of homozygous dominant cells is correlated with that of homozygous recessive cells, although induced recessive lethals are distributed independently of these, (6) a much higher frequency of variant negative colonies results from irradiation of heterozygotes ($Gg \rightarrow gg$) than from irradiation of positive haploids ($G \rightarrow g$), and (7) the estimated frequency of induced homozygous dominant variants produced by heterozygotes ($Gg \rightarrow GG$) is higher than the frequency of induced positive reversions produced by homozygous recessive diploids ($gg \rightarrow Gg$).

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A CYTOLOGICAL METHOD FOR GENOME ANALYSIS IN GOSSYPIUM

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IN AN earlier paper (MENZEL and BROWN 1952), the behavior of a radiation-induced reciprocal chromosome translocation in *Gossypium hirsutum* L. was described. This translocation (designated 2B-1) had two salient features which promised to be of considerable convenience for further cytogenetic analyses. (1) The interchanged arms were very unequal, almost the whole arm of one chromosome ("chromosome 2") having exchanged places with a very short segment of another chromsome ("chromosome 1"). As a consequence, at metaphase I in the translocation heterozygote, an interstitial chiasma was present proximal to the point of attachment of the longer ("A") arm in about 98% of the pollen mother cells, whereas the shorter translocated ("x") end was never paired. These relationships made it possible to determine the presence or absence of chiasmata at metaphase I with a high degree of accuracy at six different positions in the IV. (2) Because of these morphological features, it was possible to demonstrate that a wide array of viable, fertile deficiency-duplication genotypes was recovered from the translocation heterozygote.

The present paper reports the use of the 2B-1 translocation in further studies designed to determine the genome affinities of the two chromosomes involved and to analyze the behavior of corresponding chromosomes introduced into *G. hirsutum* from its diploid relatives.

Cytological analyses were made from temporary iron-acetocarmine smears of pollen mother cells. To facilitate discussion and illustration, numerals or letters have been assigned to chromosomes and chromosome ends and segments involved in the studies reported here. It should be emphasized that the system adopted here is provisional, and not intended as a permanent scheme for the genus. The latter must await the accumulation of additional stocks and more complete data.

GENOME AFFINITIES OF CHROMOSOMES 1 AND 2 OF THE 2B-1 TRANSLOCATION

It has been amply demonstrated that the amphidiploid species of Gossypium, including *G. hirsutum*, have one set (or subgenome) of 13 chromosomes similar to those of the wild American diploid species of Gossypium, while the other subgenome of 13 chromosomes is similar to those of the diploid Asiatic cultivated species. Following BEASLEY (1940), the genome symbol $(AD)_1$ designates the haploid chromosome set of *G. hirsutum*, while A and D refer, respectively, to the Asiatic cultivated and the American wild species. The two diploid species employed in the present study were *G. raimondii*, D_5, and *G. herbaceum*, A_1. Subgenomes of *G. hirsutum* are referred to separately as A_h and D_h (BROWN and MENZEL 1952b).

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When *G. hirsutum* is crossed with a D-genome species, the F_1 hybrid characteristically forms 13 small D II's and 13 A_h I's at metaphase I, with a low frequency of III's, averaging less than one per cell, due principally to pairing of A_h chromosomes with D II's (for review, see BROWN and MENZEL 1952a). Similarly, when *G. hirsutum* is crossed with an A-genome species, the A and A_h chromosomes pair, and the D_h chromosomes remain largely unpaired (reviewed by GERSTEL 1953).

It is possible, therefore, to assign *hirsutum* chromosomes which are marked by translocations to their respective subgenomes by crossing a plant carrying the trans-

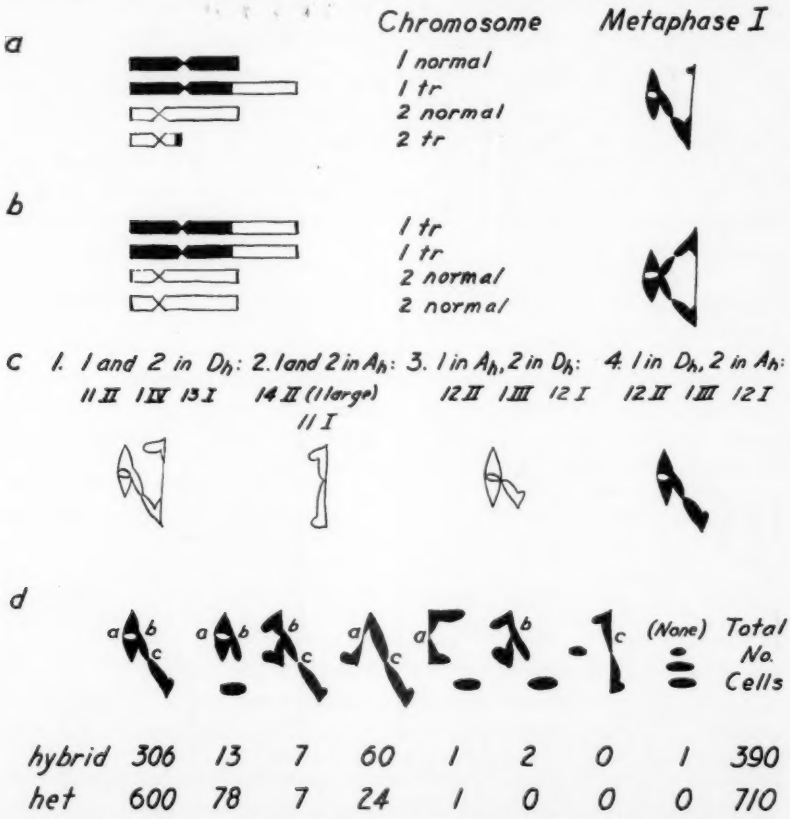
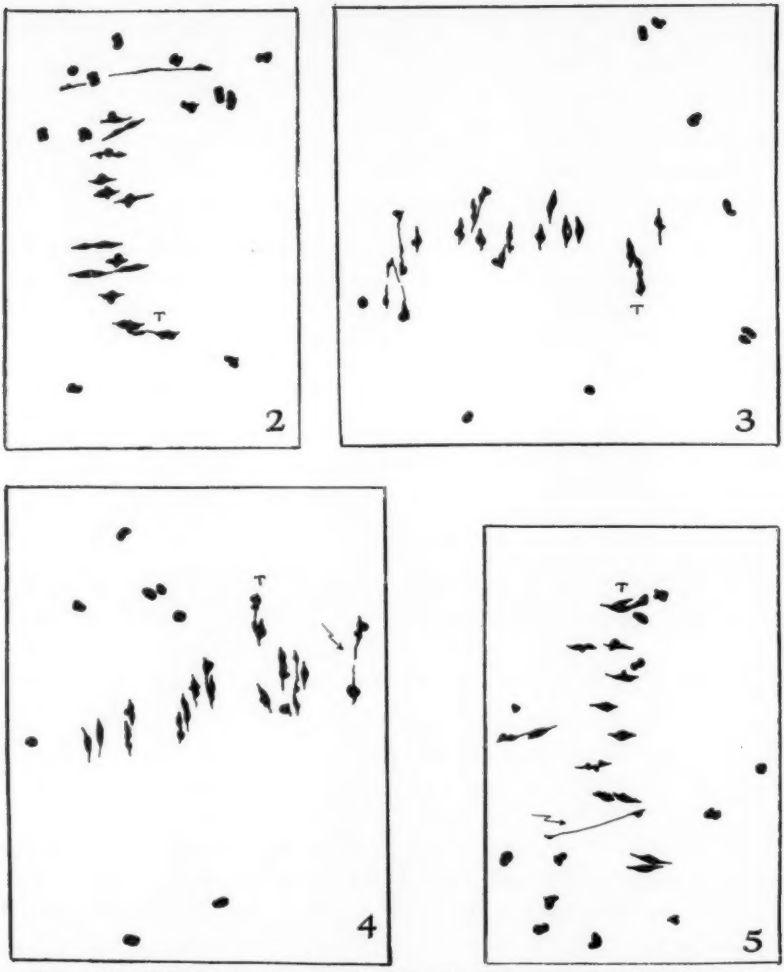

Chromosome
Metaphase I
a
1 normal
1 tr
2 normal
2 tr
b
1 tr
1 tr
2 normal
2 normal
c 1. 1 and 2 in D_h: 2. 1 and 2 in A_h: 3. 1 in A_h, 2 in D_h: 4. 1 in D_h, 2 in A_h:
11 II 1 IV 1 3 I
14 II (1 large)
1 I
12 II 1 III 1 2 I
12 II 1 III 1 2 I
d
a b c
(None)
Total No. Cells
hybrid 306 13 7 60 1 2 0 1 390
het 600 78 7 24 1 0 0 0 710

FIGURE 1. a. Chromosome structure and MI configuration of the heterozygous 2B-1 translocation. b. Chromosome structure and MI configuration of the 2n + 2A − 2x deficiency-duplication dwarf. c. Identifying configurations expected in the F_1 hybrid 2n + 2A − 2x × *G. raimondii* for the four possible genome affinities of chromosomes 1 and 2 of the 2B-1 translocation. Contingency number 4 proved to be the correct one. d. Frequency of translocation III's observed in the triploid hybrid and in the translocation heterozygote with various combinations of chiasmata at positions a, b, and c. In the case of the heterozygote, the fourth possible chiasma (see fig. 1a) is ignored.



FIGURES 2–5. Metaphase I configurations in pollen mother cells of the triploid hybrid 2n + 2A − 2x × *G. raimondii*. The III which is due to the translocation is marked "T" in each figure. The other III's are due to intergenomic ($D_hD_sA_h$) association.

FIGURE 2. A typical cell with 12 II 1 III 12 I.

FIGURE 3. The maximum association seen: 9 II 4 III 9 I.

FIGURE 4. 12 II 2 III 9 I. The arrow indicates a loose II composed of two A_h chromosomes.

FIGURE 5. 10 II 2 III 13 I. The arrow indicates a dividing, or misdividing, I. One of the potential D_sD_h II's has failed to form and is represented by two I's smaller than the rest.

located chromosomes to either an A or a D species, or to both, and comparing the pairing in the F_1 hybrids with that in hybrids carrying the normal *hirsutum* arrangement.

In practice, however, the analysis is limited to (AD)D hybrids for two reasons. (1) Hybrids between *G. hirsutum* and the two A-genome species, *G. arboreum* and *G. herbaceum*, are very difficult to obtain, while *G. hirsutum* crosses readily with several of the D-genome species. (2) It has been shown (GERSTEL 1953, MENZEL and BROWN 1954) that A_1, A_2 (*arboreum*) and A_h differ in the end arrangement of some of their chromosomes, so that $(AD)_1A_1$ F_1 hybrids form 9 II 2 IV, and $(AD)_1A_2$ F_1 hybrids form 8 II 1 IV 1 VI, instead of 13 II. Hence analysis of newly rearranged chromosomes in *hirsutum* by means of such hybrids would be complicated by the associations of IV and VI already present.

To determine the genome affinities of the chromosomes involved in the 2B-1 translocation, a cross was made between a derived deficiency-duplication dwarf of the genotype 2n + 2A − 2x (MENZEL and BROWN 1952) and *G. raimondii*. Figure 1 shows diagrammatically the chromosome structure of the 2B-1 heterozygote (figure 1a) and of the dwarf parent used in the cross (figure 1b), and the identifying configurations expected in the F_1 hybrids for the four possible genome affinities (figure 1c), namely if (1) both chromosome 1 and chromosome 2 were in the D_h subgenome, (2) both chromosomes were in the A_h subgenome, (3) chromosome 1 were in the A_h and chromosome 2 in the D_h subgenome, or (4) chromosome 1 were in the D_h and chromosome 2 in the A_h subgenome.

Pairing in the F_1 hybrids, illustrated in figures 2–5, conformed with the fourth contingency; that is, chromosome 1 is in the D_h subgenome and chromosome 2 is in the A_h subgenome. Pairing was analyzed in 390 cells of the hybrids. A total of 436 III's were seen, an average of 1.12 per cell. Of these, 63 (0.16 per cell) were due to DDA III's and 373 were due to the translocation. No difficulty was encountered in distinguishing the two types of trivalents by means of the long translocated A arm in the latter. The two types of trivalents are illustrated in figures 2–5. The two chromosomes 1 were paired in one or both arms in all but one cell. In a single cell a loosely-paired II composed of two A_h chromosomes was seen (fig. 4). In another cell (fig. 5), division or misdivision of an A_h I appeared to be in progress.

COMPARISON OF CHROMOSOME 1 OF D_5 AND CHROMOSOME 1 OF D_h

The ease with which the translocation III could be recognized in the triploid hybrids made it possible to compare the frequencies of chiasmata at three positions (designated a, b, and c, fig. 1d) in the III at metaphase I in the triploid, where the normal chromosome 1 was from D_5 and the altered chromosome 1 from D_h, with the frequencies of chiasmata at the same positions in the translocation heterozygote, where both normal and altered chromosome 1 were from D_h. Figure 1d indicates the frequency of configurations due to various combinations of the three chiasmata in the two types of plants.

Table 1 shows the frequencies of each of the three chiasmata in the hybrid and in the translocation heterozygote. A chi-square analysis of these data was carried

TABLE 1

Frequency of chiasmata at positions a, b, and c in (I) the 2B-1 heterozygote and (II) the 2n + 2A − 2x × D_b triploid hybrid

Date of sample	Frequency of chiasmata at						Total PMC
	a		b		c		
	No.	%	No.	%	No.	%	
I							
Summer 1951	139	*100.0*	137	*98.6*	118	*84.9*	139
Summer 1952	145	*97.3*	138	*92.6*	122	*81.9*	149
Summer 1953	294	*99.7*	286	*96.9*	269	*91.2*	295
Winter 1953–54	127	*100.0*	122	*96.1*	122	*96.1*	127
Total	705	*99.3*	683	*96.2*	631	*88.9*	710
II							
10–1–53	102	*99.0*	87	*84.5*	98	*95.1*	103
11–13–53	196	*98.0*	168	*84.0*	193	*96.5*	200
11–13–53	30	*93.7*	28	*87.5*	30	*93.7*	32
11–16–53	52	*94.5*	44	*80.0*	51	*92.7*	55
Total	380	*97.4*	327	*83.8*	372	*95.4*	390

	x^2 (1 d.f.)	P
aI vs. aII	4.11	<0.05
adjusted	3.15	>0.05
bI vs. bII	52.91	<0.01
cI vs. cII	14.49	<0.01

out by Dr. M. J. Garber of the Department of Genetics, A. and M. College of Texas.

The frequency of chiasmata at position a, in the unaltered arm of chromosome 1, does not differ greatly between the *raimondii* hybrid and the heterozygote. It was present in 97.4% of PMC's in the former and 99.3% in the latter. The adjusted chi-square of 3.15 is not significant.

The frequency of the interstitial chiasma proximal to the point of interchange, position b, on the other hand, was consistently *lower* in the triploid (83.8% of PMC's in the triploid, 96.2% in the heterozygote). The chi-square value of 52.91 is highly significant.

It appears, therefore, that when a D_b chromosome 1 is substituted for the normal D_h chromosome in the configuration, chiasmata are reduced significantly in the "right" arm but not in the "left" arm.

Comparison was also made of the chiasma frequency at position c, between the translocated and the normal arm of chromosome 2. Since these segments were from A_h in both cases, no difference in chiasma frequency was expected. However, the frequency was found to be appreciably *higher* in the triploid (95.4%) than in the heterozygote (88.9%). The difference is statistically significant.

It will be noted that chiasmata are reduced at b and increased at c in the hybrid as compared with the heterozygote. This suggests that there is some interference or competition between b and c in the translocation heterozygote. When this interference is reduced as a consequence of the lower chiasma frequency proximal to the point of interchange in the right arm when a D_5 and a D_h chromosome 1 are present together, chiasma formation at c rises accordingly.

COMPARISON OF CHROMOSOME 2 OF A_h WITH CHROMOSOME 2 OF A_1

Because of the crossing difficulties mentioned above, it was not feasible to compare chromosome 2 of 2B-1 directly with its A_1 homologue in a triploid F_1 hybrid. However, GERSTEL (1953) has shown that the *hirsutum-herbaceum* F_1 hybrid characteristically forms 2 IV at metaphase I, due to different end arrangements of four chromosomes in A_1 and A_h. The A_1 translocation complexes may be introduced into fertile, 52-chromosome plants by first doubling the F_1 hybrid with colchicine, and then backcrossing to *G. hirsutum* until aneuploidy has been eliminated (MENZEL and BROWN 1954). Some of the resulting 52-chromosome plants have one or both of the IV complexes. One such plant (Z850), which had metaphase pairing of 24 II 1 ring IV, was crossed with a plant homozygous for the 2B-1 end arrangement. Cytological analysis of ten F_1 plants gave the following results:

(1) Five plants had only the heterozygous 2B-1 configurations and received only normal A_h chromosomes from Z850.

(2) Two plants formed a figure of VI in most cells, proving that chromosome 2 of 2B-1 is one of the chromosomes involved in the IV of Z850. In other words, chromosome 2 is one of the 4 A_h chromosomes which differ in end arrangement from the chromosomes of A_1. These two plants received two chromosomes with the A_1 end arrangement from Z850.

(3) Three plants showed configurations different from either of the above types. They conformed with the pairing expected if two complementary deficiency-duplications were functioning from the IV complex in Z850.

The maximum configurations expected if the two balanced and four numerically equal unbalanced gametes from Z850 function are shown in figure 6. The arms of the A-genome differential chromosomes have been assigned numbers from 1 to 10 (MENZEL and BROWN 1954). At present we do not know which of eight chromosome arms (arm 3 to arm 10) was involved in the 2B-1 translocation.[2] Figure 6 has been drawn on the assumption that chromosome 2 is chromosome 3–5 of A_h and that arm 5 is the translocated or A arm. The configurations would be the same regardless of which of the eight arms is actually involved. The D_h chromosome 1 is designated 27–28 for convenience, with end 28 corresponding to the short, non-pairing translocated or x segment. Two of the deficiency-duplications recovered corresponded to type 5 of figure 6 and one to type 3.

Insufficient data have been obtained to allow a comparison of chiasma frequencies when an A_1 chromosome is substituted for the A_h chromosome 2 in the configuration.

[2] Since this paper was prepared, further studies have shown that chromosome 2 of the 2B-1 translocation must be either chromosome 3-5 or chromosome 4-6 of A_h.

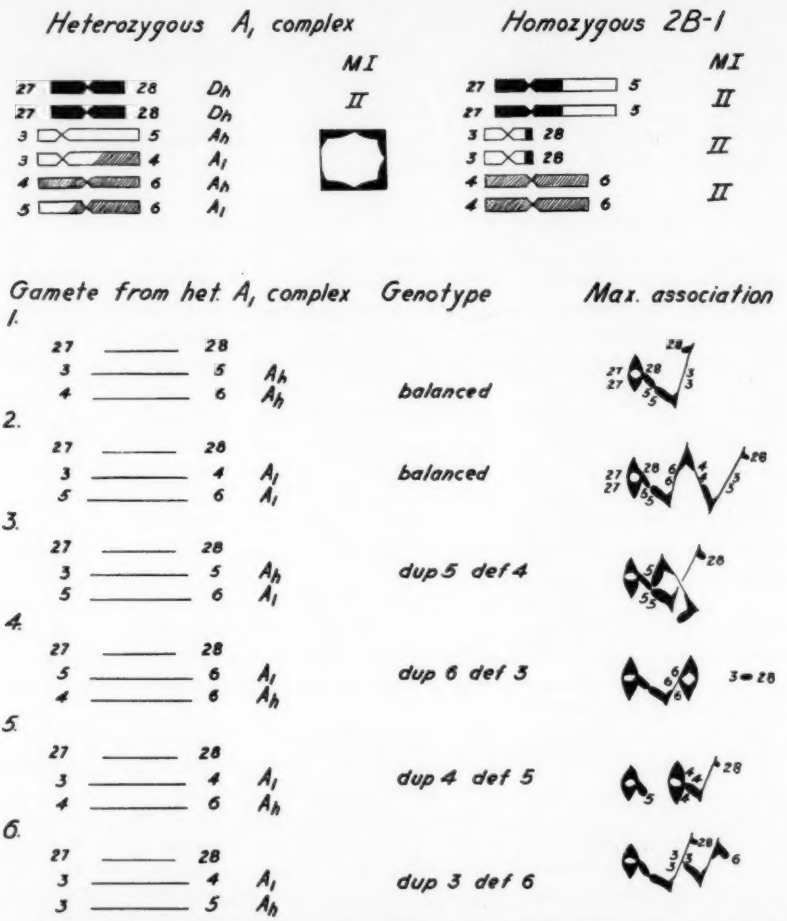


FIGURE 6. The chromosome end arrangements of Z850, which is heterozygous for two of the A_1 differential chromosomes; the end arrangements of plants homozygous for the 2B-1 translocation; the six genotypes expected if all the balanced gametes and the four numerically equal nondisjunctional gametes all function in a cross between the two; and the maximum metaphase association expected for each of the six genotypes. This diagram is based on the assumption that chromosome 2 of the 2B-1 translocation is differential chromosome 3-5 of A_h, and that end number 5 was the translocated end. The normal end arrangement of *G. hirsutum* in this scheme is 3-5, 4-6, 27-28.

However, even if such a comparison were made it would not be comparable to the study of chromosome 1, since after several generations of backcrossing a large portion of the content of the original A_1 arm may have been replaced by A_h segments, even though the A_1 end arrangement has been retained.

DISCUSSION

The differences in end arrangement existing in nature among the A_1, A_2 and A_h genomes are of particular interest because of the meiotic complexities they produce in three-species tetraploid hybrids of the genome constitution (AD)AD. Several such hybrids are being used as a source of primary breeding stocks at this station. Inconclusive data from this material (MENZEL and BROWN 1954, BRADFORD 1954, A. M. MEMON unpublished) had suggested that deficiency-duplications are recovered from the A-genome multivalent complexes. But analysis in the three-species hybrid material was impaired by the lack of morphologically marked chromosomes and by the occurrence in such material of an unexpectedly high frequency of excess multivalents attributed to intergenomic (AD) pairing. The present study demonstrates conclusively that both duplications and deficiencies for at least two ends of the five differential chromosomes are functional. It should eventually be possible, with other suitable translocation lines, to analyze in considerable detail both the dosage effects and the genetic contents of the differential A-genome chromosomes.

Substitution of a D_5 for a normal D_h chromosome 1 in the 2B-1 translocation configuration lowered the chiasma frequency little or not at all in the left arm (at position a), but appreciably in the right arm (at position b). The evidence of interference in the right arm between chiasmata proximal and distal to the point of translocation (positions b and c) does not alter the conclusion that the D_5 chromosome differs in some way from the D_h chromosome in the right arm, and that the difference is in the direction of less frequent chiasma formation when the two are present together. It does, however, introduce a doubt as to whether the *lack* of difference between the D_5 and D_h left arm is real, or only apparent because of a lack of competition. If a parallel experiment were devised in which the left arm of the D_h chromosome bore a translocated segment, a greater degree of difference might become apparent. On the other hand, the chromosomes of D_5 do pair regularly with those of D_h, hence are assumed to have a high degree of homology with them, and it is not unlikely that such differences as do exist may be unevenly distributed along the various chromosome arms.

STEPHENS (1949, 1950) has suggested that cryptic structural differences (STEBBINS 1945; STEBBINS, VALENCIA and VALENCIA 1946) exist between the chromosomes of closely related genomes of Gossypium species whose F_1 hybrids show no gross structural differences or marked non-homology at meiotic metaphase. A critical test of this hypothesis has not been readily forthcoming. BROWN (unpublished) has shown that pachytene pairing cannot be considered a criterion of chromosome or genome differentiation in Gossypium. Pachytene pairing was as intimate in both sterile and fertile F_1 species hybrids as within species, and was equally complete in all hybrids examined regardless of the degree of metaphase association. BROWN concludes, therefore, that the number of chiasmata persisting until metaphase I is still the best criterion of similarity between different genomes. The pertinent conclusion from the present data would seem to be that chromosome 1 of D_5 is not perfectly homologous with chromosome 1 of D_h, despite the fact that they were paired with each other at metaphase I in all but one out of 390 cells examined. In a formal sense, these data may be considered as evidence for cryptic structural differences between the

chromosomes of the two species, although the real nature of the differences is still obscure. If chiasmata represent crossovers, we may conclude, as STEPHENS (1949) has suggested, that the effect of the differentiation, when the two are combined in a hybrid, will be to reduce crossing over in the chromosome segments concerned, with consequent tendency of the parental characteristics determined by genes on those segments to remain more tightly linked than would be expected within either species. With appropriate translocation lines, it should be possible to test in a similar manner the differentiation of each D_h chromosome from its homologues in D_5 and in several other D genomes.

A low incidence of III's due to DDA pairing rather than to the translocation was found in the *hirsutum-raimondii* hybrid. It was mentioned above that intergenomic pairing was unexpectedly high in (AD)AD three-species hybrids. It has been suggested (MENZEL and BROWN 1954) that this pairing is not at random, but rather confined to specific chromosomes. Some 63 instances of AD chiasmata were found in the 390 cells examined (usually only one, with a maximum of three, per cell). Had any of these been chiasmata between chromosome 1 and an A_h chromosome, the resulting IV would have been readily detectable, but no such configuration was found. Hence it may be said that if this chromosome ever pairs with an A_h chromosome, it does so very infrequently.

The present studies illustrate only two of the ways in which the cytogenetic anatomy of Gossypium genomes may be explored and compared by the use of morphologically marked chromosomes. Further progress will be limited by the ease with which other lines having the morphological convenience of 2B-1, or producing a similar array of deficiency-duplication genotypes, or both, can be obtained. Earlier work (BROWN 1949) and preliminary experiments with X-ray treatments to be reported elsewhere indicate, however, that 2B-1 is not unique in either respect.

SUMMARY

The 2B-1 translocation in *G. hirsutum* consists of an interchange between a very short segment of a chromosome in the D_h subgenome (chromosome 1) and a long segment from a chromosome in the A_h subgenome (chromosome 2).

Chromosome 1 of *G. raimondii* (D_5 genome) shows a reduced frequency of metaphase chiasmata with its *hirsutum* homologue in the right arm but not in the left arm.

Chromosome 2 proved to be one of the 4 A_h chromosomes which differ from the A_1 genome (*G. herbaceum*) in end arrangement. It was demonstrated that when one of the A_h-A_1 IV complexes is introduced into *G. hirsutum* at least two types of deficiency-duplication gametes function in addition to the two types of balanced gametes.

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THE GENETIC BASIS OF A NUTRITIONAL REQUIREMENT IN DROSOPHILA[1]

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WITH the accomplishment of a technique for raising Drosophila under aseptic conditions on a synthetic medium (SCHULTZ, ST. LAWRENCE, and NEWMEYER 1946; HINTON, NOYES, and ELLIS 1951) it was possible to detect mutations that affect the nutrition of the organism. Studies of genetic changes affecting metabolic pathways have been restricted, for the most part, to microorganisms. The first change in nutritional requirement in Drosophila was reported in 1950 (HINTON and ELLIS) and described in detail in 1951 (HINTON, ELLIS, and NOYES). The nature of the change was an inability of the organism to grow and develop in the absence of the adenine component of nucleic acid in the diet; whereas a wild-type strain (Oregon-R) of Drosophila did not require nucleic acid or any of its derivatives. The present report presents data concerning the genetic nature of this nutritional requirement.

The requirement was present in a strain (*In(2LR)40d/Cy*) which contains an interbrachial inversion in the second chromosome having one break in heterochromatin. The rearrangement produces a position effect phenotype causing various abnormalities of the eye (HINTON and ATWOOD 1941; HINTON 1950). Preliminary investigations suggested that there was a correlation between the requirement and the position effect (HINTON and ELLIS 1950). Subsequent investigations indicated that if a correlation exists, it is not a direct one (HINTON, ELLIS, and NOYES 1951). It was also reported that the requirement usually, but not always, behaved as a dominant effect. This unorthodox behavior was investigated further (HINTON and ROBERTS 1952) and confirmed.

TECHNIQUE

The media used in the various experiments reported here were prepared as described previously (HINTON, NOYES, and ELLIS 1951) except that the basal medium (table 1) contained no nucleic acid or nucleic acid derivatives. In addition, some experiments were performed using a basal medium prepared by the Difco Laboratories and patterned after medium no. 4 as reported by HINTON, NOYES, and ELLIS (1951). Adenine (as ribose adenylic acid or ribonucleic acid) was added to these when necessary for control series.

Since the second chromosome inversion carried by the requiring strain is lethal when homozygous, it is balanced using the "Curly" inversion (*In(2L)Cy, In(2R)Cy*). Due to the lethals involved, 50% of the eggs fail to develop into active larvae. In order to eliminate the necessity for inoculating twice the number of eggs needed to

[1] This study was made under a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

TABLE 1

Basal medium

	Mg/Ml		Micrograms/Ml
L-arginine	0.559	biotin	0.02
L-cysteine	0.480	B_{12}	0.028
L-glutamic acid	4.418	Ca-pantothenate	6.0
glycine	1.745	choline chloride	75.0
L-histidine	0.484	pteroyl glutamic acid	36.0
L-isoleucine	1.260	pyridoxine	3.0
L-leucine	2.345	riboflavin	2.4
L-lysine	1.337	thiamine	1.5
DL-methionine	0.339	niacinamide	10.0
L-phenylalanine	1.008		*Mg/Ml*
DL-threonine	0.756	$MnSO_4 \cdot 4H_2O$	0.0129
L-tryptophane	1.745	$MgSO_4 \cdot 7H_2O$	0.246
DL-valine	1.355	$FeSO_4$	0.0129
sucrose	7.5	KH_2PO_4	0.606
cholesterol	0.4	K_2HPO_4	0.606
agar	20.0	NaCl	0.0129
		$CaCl_2$	0.0129

produce a given number of larvae, the following procedure was used: adult flies were taken from standard Drosophila cultures and placed in empty half-pint milk bottles which were inverted over flat dishes containing cornmeal, molasses, agar, and growing yeast. The adult females were allowed 12 to 18 hours to deposit eggs on this mixture. At the end of the egg-laying period the eggs were picked or brushed from the surface of the collecting dishes and placed in small baskets made of fine copper mesh. The baskets were then transferred through a series of washes with Ringer's solution and finally 70% alcohol where they remained for 45 minutes. This treatment has proven adequate to free the eggs from all bacteria and spores and yet not injure the eggs. Using sterile technique, each basket was placed into a small sterile petri dish containing a layer of agar gel. Each basket contained approximately 50 eggs. The eggs were allowed 24 hours to hatch. After the young larvae emerged from the eggs they would usually crawl through the basket onto the surface of the sterile agar. It was a simple matter to remove the larvae, on the end of a wire loop, and transfer them from the petri dish to previously autoclaved test tubes of synthetic medium. Standard bacteriological techniques were used throughout. About 20 larvae were transferred to each test tube.

The results were tabulated in terms of the percent of larvae inoculated that pupated, the average time in days required to reach the pupal stage, and the percent of the pupae which eclosed. The average time to pupation represents the most sensitive criterion for measuring the effect of the medium on growth.

CONTROL DATA

The wild-type strain (Oregon-R), used throughout, does not require adenine for complete growth and development, but growth proceeds at a slower rate when it is omitted from the medium. This is shown by the data in table 2. In the case of Ore-

TABLE 2

Typical examples of growth of the controls

Strain	Medium	Adenine in medium	Original no. larvae	Percent larvae to pupate	Av. time to pupation in days	Percent pupae to become adults
Ore′ R	lab	yes	108	97.2	12.5	97.2
		no	96	84.4	17.9	96.3
Ore′ R	Difco	yes	57	100.0	11.8	56.1
		no	232	85.3	16.9	(2)
In(2LR)40d/Cy	lab	yes	40	80.0	13.7	93.7
		no	280	9.7* av.	21.5–26.0	(22)
		yes†	146	94.5	14.3	95.6
		no†	123	6.5	21.9	(7)
In(2LR)40d/Cy	Difco	yes	32	62.5	12.4	100.0
		no	170	0.0	—	0.0

* Experiment divided into 7 parts in order to see variability: 0.0, 4.4, 2.2, 14.6, 2.1, 18.7, 25.6.
† A stock made from adults that appeared on a no-nucleic acid medium.

gon-R without adenine, pupation is delayed to around the 17th day, whereas in the *In(2LR)40d/Cy* strain usually no pupae are formed, but this is variable (table 2). When an occasional larva lives long enough to pupate, pupation does not occur until after the 20th day (table 2).

The medium manufactured by Difco allows no pupation in the *In(2LR)40d/Cy* strain unless adenine is added to it (table 2). Therefore most of the experiments were run using Difco medium. If doubt existed as to the validity of earlier experiments they were repeated on Difco medium.

A stock of *In(2LR)40d/Cy* was obtained from the exceptional flies that had developed on medium devoid of adenine. When growth of the new stock was tested, with and without adenine, there was no increase in the percentage pupation (table 2), indicating that genetic modifiers or reverse mutation are not responsible for the occasional survivor.

It should be noted that Ore′-R has a lowered adult hatch when raised on the Difco medium supplemented with adenine. Practically no adults emerge when adenine is omitted. This is despite the fact that the percent pupation is as high as when the medium is made in our laboratory, and adults form within the pupal case. The point is pertinent to some data brought forth by the experiments to be reported here.

RESULTS AND ANALYSIS

The first crosses were made in order to observe the segregation of the requirement with other marked chromosomes so that it might be located as to linkage group. Thus *In(2LR)40d/Cy* (the strain having the requirement) was crossed to a wild-type strain, Oregon-R. Eggs were collected and larvae inoculated onto media part of which contained adenine and part of which did not. Two types of flies would be

TABLE 3

*Offspring from the cross: In(2LR)40d/Cy × Ore'-R**

Adenine	Original no. larvae	Percent pupation	Av. time to pupation in days	Percent pupae becoming adults	No. adults classified	
					Inv.	Cy
Yes	438	94.8	12.0	93.0	—	—
No	363	81.5	17.5	82.8	116	116

* The combined results of 3 experiments.

expected to develop—half should have the rough eye characteristic of the *In(2LR)40d*; half, the curly wing characteristic of *Cy*. However, if the requirement is located on the *In(2LR)40d* chromosome, without adenine only Curly flies should develop; or only straight winged flies if the requirement is on the *Cy* chromosome. It might be expected that the requirement would be found on one of the second chromosomes since they are the only chromosomes characteristic of the strain, the other chromosomes having been replaced several times in outcrossing.

The results of the cross to wild-type were unexpected. Both straight wings and curly wings appeared among the offspring and with an equal frequency, even when raised without nucleic acid. The results from three separate experiments are presented in table 3. There was no significant difference when the female parent was from the Oregon strain or from the *In(2LR)40d* strain.

Not only did both types survive without adenine, but the average time to pupation was the same as it was for the non-requiring wild-type (see table 2). It can be concluded from this that neither of the second chromosomes from the requiring strain expresses the requirement when heterozygous with wild-type second chromosomes.

To see if the requirement would return when the original second chromosomes were brought back together, the offspring from the preceding cross were mated together (*Cy* of one sex × *In(2LR)40d* of the opposite sex). Since both types of flies were heterozygous for wild type chromosomes, four types of second chromosome combinations would be expected in the offspring: wild/wild, wild/*Cy*, wild/*In(2LR)40d*, *In(2LR)40d/Cy*. One of these represents the original combination of second chromosomes characteristic of the strain with the adenine requirement. The results of four such crosses were inconclusive, since the figures were small and some of the other classes were as small as the class under inspection.

In light of these inconclusive data it was deemed best to try a different approach. Therefore, a series of stocks was synthesized representing various combinations of second and third chromosomes from the Oregon wild and the strain with the requirement, and in various cytoplasms.

The stocks synthesized are listed below. The following abbreviations and symbols were employed to refer to the chromosomes: *In*—the second chromosome carrying the Inversion *(2LR)40d*; *Cy*—the 2nd chromosome with the Curly inversions; *Ore'*—the wild-type 2nd chromosome from the Oregon-R stock; *I*—the unmarked 3rd chromosome from the stock of *In(2LR)40d/Cy*; *O*—3rd chromosome from the Oregon-R stock; *Bl*—unmarked 3rd chromosome from a stock of Bristle Lobe/*Cy*.

Stocks synthesized

Stock code no.	Chromosomes		Cytoplasm from	Notation
	2nd	3rd		
R_1	*In/Cy*	*I/I*	*In(2LR)40d/Cy*	Combination recovered following an outcross to *Cy/Pm; D/Sb.*
D_4	*In/Cy*	*I/I*	*Cy/Pm; D/Sb*	
R_4	*In/Cy*	*I/I*	Oregon-R	
R_2	*In/Cy*	*0/0*	Oregon-R	
D_2	*In/Cy*	*Bl/Bl*	*Cy/Pm; D/Sb*	
R_3	Ore'/'Ore	*I/I*	*In(2LR)40d/Cy*	

The stocks were synthesized using the technique of crossing flies bearing desired chromosomes to a stock containing 2nd and 3rd chromosomes which were marked by dominant traits and crossover suppressors. By proper crosses, the desired combinations of chromosomes were brought together and the dominant markers eliminated. The dominant marker stock used was *Cy, sp^2/Pm, dp b; DcxF, ru h ca/ In(3R)Mo, Sb.*

The newly synthesized stocks were next raised on the synthetic medium in order to learn which combinations of the chromosomes and cytoplasm manifested the adenine requirement. The results are summarized in table 4.

Taking only the percent pupation as the criterion for the presence or absence of the adenine requirement, it can be seen that the 2nd chromosome and not the 3rd is necessary for the requirement to be expressed. In the case of R_2 and D_2, where the 3rd chromosomes of the requiring strain have been replaced by 3rds from the Oregon and *Bl L* stocks respectively, the percent pupation remains close to zero. In fact, the second chromosomes are the only genetical factor shared in common by R_2, D_2, and the original requiring strain. Conversely, when the 3rd chromosome and cyto-

TABLE 4

The adenine requirement of stocks R1-4 and D2-3

Stock	Adenine in medium	Original no. larvae	Percent larvae to pupate	Av. time to pupation in days	Percent pupae to become adults
R_1	yes	63	71.4	15.4	100.0
	no*	299	33.1	23.1	97.0
R_2	yes	23	73.9	13.5	0.0
	no	89	0.0	—	0.0
R_3	yes	42	85.7	11.6	97.2
	no	103	59.2	17.6	98.3
R_4	yes	103	66.0	13.3	75.0
	no	221	14.0	20.8	38.7
D_2	yes	84	88.1	13.7	86.5
	no	196	1.5	17.0	(1)
D_4	yes	74	55.4	14.3	80.5
	no	209	4.3	21.6	(3)

* Medium was such that some larvae with the requirement will pupate in the absence of adenine, but not until after the 20th day, as discussed in the section on TECHNIQUE.

plasm of the original strain are left unaltered, but the 2nd chromosomes are replaced (R_3), the requirement is lost.

R_1, D_3, and R_4 are alike in respect to the second and third chromosomes, but have different cytoplasms. There is some pupation in each case regardless of the medium used. Although this may not be significant it is possible that during the outcrossing, from which R_4 and D_3 were derived, genetic modifiers were acquired. On the other hand, R_2 and D_2 had a similar origin, yet they do not pupate on the Difco medium. The one thing that R_4 and D_3 have in common which differs from that which R_2 and D_2 have in common are the third chromosomes. R_4 and D_3 both have third chromosomes from the original *In(2LR)40d/Cy* stock while R_2 and D_2 both lack such third chromosomes. This suggests that the third chromosome in the original requiring strain carries modifiers of the requirement which can operate when in certain cytoplasm or when the flies are grown on certain media.

Thus it seems fairly certain that the basis for the adenine requirement is a condition of a second chromosome in the stock *In(2LR)40d/Cy*. The third chromosomes of this stock may possibly carry factors capable of modifying the requirement under certain conditions.

Finally, it should be noted (table 4) that in the case of stock R_2, no adults appeared either in the presence or absence of adenine. In part this is reminiscent of the behavior of Oregon-R on the Difco medium (table 2). There, no adults appeared when adenine was omitted from the medium even though they did appear when it was added. The only thing unique about R_2, in comparison with Oregon-R, is that the second chromosomes have been replaced by seconds from the *In(2LR)40d/Cy* stock. This suggests that the second chromosome of Oregon-R is able to perform some vital function, when adenine is in the diet, that neither the third of Oregon nor the seconds of *In(2LR)40d/Cy* can perform. Thus in the combination of seconds from *In(2LR)40d/Cy* and thirds from Oregon, this system concerned with adult eclosion fails to function regardless of the adenine.

Before it was realized that the *In(2LR)40d/Cy* stock required adenine, HINTON (1949) studied the eye phenotype characteristic of the *In(2LR)40d* chromosome. The variable degree to which the eye is affected was measured by distribution curves. It was found that various factors could shift the malformation in one direction or the other. When *In(2LR)40d/Cy* was crossed to Oregon-R and the offspring heterozygous for the inversion and Ore′-R were studied, it was found that the distribution curve had shifted radically toward the extreme effect. This was not true in the case of some of the other crosses made, the curve remaining stationary. The latter was particularly true in the case of a cross to a stock *Bl* L^2*/Cy* (Bristle, Lobe) when the *In(2LR)40d/Cy* offspring were compared with the *In(2LR)40d/Cy* parental stock. Further work was presented to show that the presence or absence of the *Cy* chromosome was not responsible for the change in degree of effect and it was concluded that some third chromosome factor was acting to modify the phenotype.

Since the Oregon-R stock modified both the effect upon the eye and the adenine requirement, and if these two are related, it should follow that the *Bl* L^2*/Cy* stock should not modify the adenine requirement, since it did not modify the eye effect. Therefore a study was made of the adenine requirement of the offspring of a cross of

TABLE 5

Offspring from four crosses of Bl L^2/Cy × In(2LR)40d/Cy raised without adenine

	Original no. larvae	Number of pupae by 21st day	Number of adults
1	70	3	1
2	23	1	0
3	390	0	0
4	177	3	2

Bl L^2/Cy × *In(2LR)40d/Cy*. Three types of offspring would be expected: *Bl L^2/In(2LR)40d*, *Bl L^2/Cy*, *In(2LR)40d/Cy*. However, practically no offspring of any type survived without adenine (table 5), although all three types were obtained in large number in the control with adenine.

The results indicate that not only does the requirement remain after this outcross (as opposed to the results when Ore′-R was used in the outcross) but that the *Bl L^2/Cy* stock also requires adenine.

In order to obtain further evidence in this connection, *In(2LR)40d/Cy* was crossed to Ore′-R and the noncurly offspring (*In(2LR)40d*/Ore′) were selected and mated to *Bl L^2/Cy*. Four types of offspring would be expected: *In(2LR)40d/Bl L^2*, *In(2LR)40d/Cy*, *Bl L^2*/Ore′, *Cy*/Ore′. Without adenine in the medium the only type that survived was the *Cy*/Ore′ which is the only type lacking both the *In(2LR)40d* chromosome and the *Bl L^2* (17 obtained from 227 larvae). This confirms the results of the previous crosses and further shows that the requirement on the *Bl L^2* chromosome is dominant to Oregon.

Examining all of the foregoing evidence, the suggested conclusion is that the basis of the adenine requirement in the *In(2LR)40d/Cy* strain is genetically characteristic of the *In(2LR)40d* second chromosome, is dominant to the Curly second chromosome, but is recessive to the Oregon second chromosome. If this is true, then the question arises as to whether the genetic basis of the requirement is a mutant gene, a position effect of the chromosomal inversion which the strain contains, or some other factor.

In an attempt to distinguish between these possibilities, a study was made of the adenine requirement of a series of stocks which were described by HINTON (1950) and which represent various cytological alterations of the original *In(2LR)40d*. In all of these stocks one end of the original inversion, or both ends, had been involved in a subsequent inversion, translocation, or reinversion. The earlier analysis of these stocks established that the eye effect was a position effect of the inversion. If the heterochromatin located at either end of the inversion were removed to other positions in the chromosome complement by means of these induced aberrations, the eye effect would usually disappear.

Therefore it would seem logical to expect that, if the adenine requirement and the eye effect were both position effects of the inversion, both would disappear upon alteration of the original inversion in some of the cases. A series of these stocks was raised on media lacking adenine. The results are given in table 6.

From the results in table 6 it appears that regardless of the chromosomal arrangement (and the appearance of the eye) the requirement for adenine persists. However,

TABLE 6

The adenine requirements of various stocks representing cytological alterations of In(2LR)40d

Stock	Adenine in medium	Original no. of larvae	Percent larvae* to pupate
In(2LR)IIA	yes	245	69.8
	no	366	8.2
T(2:3)IIG	yes	12	83.3
	no	16	6.2
In(2LR)IIP	yes	88	63.6
	no	230	0.4
T(2:3)IIAH	yes	13	84.6
	no	13	0.0
In(2LR)IIBI	yes	69	59.4
	no	183	1.1
In(2LR)IICA	yes	50	46.0
	no	159	3.8
T(2:3)IICK	yes	34	88.2
	no	87	25.3
In(2LR)IICQ	yes	36	75.0
	no	89	1.1
In(2LR)IICR	yes	23	73.9
	no	34	5.9
T(2:3)IICS	yes	19	78.9
	no	52	0.0
In(2LR)IICX	yes	46	60.1
	no	94	8.5
IICZ	yes	46	58.6
	no	185	0.0
T(2:3)IIDA	yes	32	75.0
	no	70	5.7
IIDC	yes	40	20.0
	no	92	0.0
In(2LR)IIDD	yes	54	66.6
	no	147	2.1
In(2LR)IIDE	yes	24	70.8
	no	103	7.8
T(2:3)IIDG	yes	36	72.2
	no	97	10.3
T(2:3)IIDJ	yes	78	67.9
	no	174	2.9

* The figures for the percent pupae to become adults are not given since they were consistently close to 100% and therefore are of no significance here.

in some cases, a few individuals reached the adult stage with adenine absent from the medium. These cannot be explained as being due to the type of medium used since the Difco medium, which allowed no growth of *In(2LR)40d/Cy*, was used throughout. It may be noted that the percentage of larvae to pupate is quite variable from stock to stock. Seven of the stocks showed no or practically no pupation while the others ranged from 2.1% up to 25.3%. There appears to be no correlation between the amount of pupation and the appearance of the eye or the type of chromosomal rearrangement.

DISCUSSION

It can be concluded that the genetic control for the adenine requirement resides on the same second chromosome as the inversion (*In(2LR)40d*). However, its exact nature has been more difficult to determine.

The original supposition that the requirement may have resulted as a position effect due to the chromosomal inversion is made untenable by the results obtained from testing the series of reversion stocks. These stocks represent cases of cytological alteration of the original inversion accompanied by the disappearance or partial disappearance of the eye effect. The eye effect may be considered a position effect of the inversion which disappears if the heterochromatin at either end of the inversion is removed. Since in no case, of the 18 different tested, had the requirement disappeared, it would follow that the requirement and the eye effect are not being caused by the same agent.

The very fact that the requirement remains in all of the reversion stocks makes the second supposition unlikely, namely that it is due to a gene mutation. If the requirement were due to a point mutation somewhere along the second chromosome it would have had ample opportunity to become lost from some of the stocks through crossing over, because of the very nature of the chromosomal arrangements and the method used to balance the stocks originally. Especially would this be true in the case of the translocations between the second and third chromosomes since they were maintained for many generations heterozygously with cytologically normal chromosomes marked with a second and a third chromosome recessive mutant (*vg*; Hn^{rI}). This method of maintaining the stocks had to be abandoned because the recessive genes in question crossed over onto the translocated chromosomes with such a high frequency as to render them useless as marker genes.

One of the initial breaks which produced the original inversion was located in a heterochromatic region comprising roughly one-third of the mitotic chromosome (HINTON 1941). This region does not expand in the formation of the salivary gland chromosomes and therefore cannot be seen and studied there. Since all cytological analyses were made using only salivary gland chromosomes, one does not know how much heterochromatin was carried to the left end of the inversion or how much remained at the right end. However, it seems definite that a relatively large section is involved at each end of the inversion since, upon irradiating further, breaks occurred at these two places with a too high frequency (HINTON 1950). It would appear that these breaks may occur anywhere within these large regions of heterochromatin and not necessarily at the exact site of the original break. Thus when a subsequent rearrangement occurs, the heterochromatin may become further subdivided instead of being moved as a block. This would also be the most likely situation in the cases which appear to be cytologically complete reinversions.

In fact, the only thing held in common by all of the stocks which have maintained the requirement is the displacement of heterochromatin from its original position in the wild-type. If this particular block of heterochromatin cannot function properly in adenine synthesis when moved from its normal position, or when subdivided, then it would not be surprising that all stocks in the reversion series still show the requirement for adenine. This is to suggest that some step in the synthesis of adenine is controlled by a region of heterochromatin and that the requirement under study

is due to a failure of that region of heterochromatin to function. This is the same region of heterochromatin which exerted the position effect just discussed.

The failure to obtain adults, in certain cases, indicated the presence of additional systems concerned with nucleic acid synthesis and utilization. These cases have been explained in purely hypothetical terms. The essential feature of the analysis is that the third chromosome of the Oregon-R strain apparently carries a factor which makes it difficult for the strain to "store" enough adenine (or perhaps nucleic acid) to last throughout the non-feeding pupal stage. The amount synthesized depends upon certain dietary conditions. The Oregon-R strain can accumulate enough to complete its development only when it is both capable of synthesizing the compound and obtaining extra amounts from the medium. The inversion strain lacks the third chromosome factor and therefore can complete its development from the adenine in the medium, even though it cannot synthesize any due to the presence of the second chromosome. Thus the combination of this third chromosome factor from Oregon-R with the second chromosome of the inversion strain prevents the completion of development even though adenine is present in the medium, as demonstrated by strain R_2.

It may well be that this third chromosome factor found in Oregon-R is the factor that enhanced the eye effect reported earlier. The degree of effect upon the eye is significantly more extreme both in the inversion offspring from the cross of *In(2LR)40d/Cy* × Ore′-R and in the flies from the strain R_2 which have Oregon third chromosomes.

The relation of the adenine requirement to the position effect phenotype as measured by the disturbance in eye formation can now be considered. Our hypothesis which grows out of the foregoing data and discussion is as follows: The original chromosomal rearrangement removed part of a relatively large section of heterochromatin from its normal position near the kinetochore. In its original position and condition this region of heterochromatin served in the synthesis of adenine. As a result of the original chromosomal rearrangement, part of this section of heterochromatin came to lie adjacent to euchromatic genes in the distal part of the left arm of the chromosome, and reciprocally, euchromatic genes from the distal part of the arm came to lie next to that part of the heterochromatin which remained in its normal position. The altered heterochromatin has a general influence on the adenine metabolism of the cell as well as a localized influence on the functioning of the euchromatic region immediately adjacent to it. This influence on the adjacent euchromatin was observed as a malformed eye. Any further disturbance of nucleic acid synthesis or utilization causes the euchromatin adjacent to the influencing heterochromatin to function still less efficiently. When either the distal section of heterochromatin is moved by subsequent chromosomal rearrangement to a new location or when the euchromatin adjacent to the section of heterochromatin remaining at the kinetochore is moved, the localized influence of the heterochromatin disappears. This suggests that the euchromatic region was originally divided into two parts, either part being capable of functioning as the whole when away from the influence of the heterochromatin. The altered heterochromatin, on the other hand, does not function normally in the synthesis of adenine, regardless of its position on the chromosomes. It is not known whether this heterochromatin, in other positions,

exerts an influence on the adjacent chromatin since tests have only recently been started to detect such a situation.

SUMMARY

A strain which has an inherited requirement for adenine was studied in an attempt to identify the genetic basis responsible.

When the strain with the requirement was crossed to a wild-type lacking any requirement for adenine, none of the offspring showed a requirement. When these offspring were inbred and their progeny studied, the data suggested that the requirement was regained only by those individuals with the original second chromosome pair from the adenineless strain, but the results were not definite.

A series of stocks was synthesized representing various combinations of second and third chromosomes from the wild-type and the strain with the requirement. Only those stocks which carried the second chromosomes of the requiring strain proved to have the requirement.

Since the two second chromosomes of the requiring strain are different both cytologically and genetically, it was necessary to make further crosses in order to identify the requirement with one or the other. The results showed that the requirement followed the chromosome which has an inversion known as *In(2LR)40d*.

The various crosses showed that the requirement behaved in a recessive manner to the wild-type but in a dominant manner to second chromosomes from certain other strains.

Attempts were made to separate the requirement from the chromosomal inversion by studying a series of cytological alterations of the inversion. Eighteen different cases were studied and it was found that in no case was the requirement lost. These 18 strains and *In(2LR)40d*, from which they were derived, have in common only the factor of transposed heterochromatin brought about through the original inversion and further transposed by the subsequent alterations. Therefore, it is suggested that the genetic basis of this nutritional requirement lies in the heterochromatin and is a property of the altered heterochromatin itself.

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THE INHERITANCE OF YELLOW-GREEN, A POSSIBLE MUTATION IN COTTON[1]

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FEW instances of duplicate factor inheritance are recorded for the allotetraploid Gossypium species. Only one of these, duplicate yellow petal factors (HARLAND 1939), is found within a single species, *G. barbadense* L., and the remainder occur in F_2 and backcrosses involving allotetraploid *G. barbadense* and *G. hirsutum* L. (HARLAND 1939). The most commonly known duplicate factor in cotton is the complementary chlorophyll deficient found by HARLAND, (summarized 1939), to occur in F_2 between certain representatives of both species. A somewhat analogous chlorophyll deficiency has been shown to occur in *Nicotiana tabacum* by CLAUSEN and CAMERON (1950) who evaluated its possible indication of diploidization in that allotetraploid species. The paucity of duplicates in *G. hirsutum* might be interpreted to support a less recent origin of the allotetraploid cottons than that suggested by HUTCHINSON, SILOW, and STEPHENS (1947). The absence of duplicate inheritance in *G. hirsutum* could also result when inheritance studies are confined within varieties derived, more or less, from a common breeding source. The inheritance of yellow-green, to be reported herein, is one such instance of duplicate factor inheritance within Upland cotton, *G. hirsutum*, which, when confined to selected varieties appears to be a recessive at a single locus and typical of genetic inheritance in this species.

DISCOVERY OF YELLOW-GREEN

MR. P. H. KIME of North Carolina State College observed several small plants having yellowish-green leaves in an F_3 population from a cross between Acala W29 and Coker 100 Upland cottons. No record was made of the genetic ratio as most of the green plants had been removed in a thinning operation. These yellow-green plants were shown to the writer concomitant with the suggestion that these did not appear to be the common virescent-yellow mutant (HARLAND 1939) of Upland cotton. When several of these yellow-green plants having dominant yellow pollen were crossed on a virescent-yellow tester, the resulting F_1 contained both green and yellow plants having a yellow pollen. These results were anomalous so the writer began a study of the inheritance of yellow-green.

DESCRIPTION OF YELLOW-GREEN

The progeny grown from seed of self-pollinated yellow-green mutants had lemon yellow cotyledons when grown inside a greenhouse and yellowish-green cotyledons of uniform color when grown under field conditions. The seedlings of yellow-green could be distinguished from those of virescent-yellow and normal stocks on the basis

[1] Contribution of U. S. Field Station, Sacaton, Arizona, Field Crops Research Branch, Agricultural Research Service, U.S.D.A.

of cotyledon color, since cotyledons of virescent-yellow have a green color similar to that of commercial varieties although under some conditions the cotyledons may be greenish with streaks of yellow. The leaves of yellow-green plants are at first yellow, then become yellow-green, and later in maturity become green and barely distinguishable from leaves of normal plants. Yellow-green plants are especially sensitive and exhibit leaf burn caused by application of elemental sulfur for insect control. Normal commercial varieties and virescent-yellow stocks are insensitive and do not burn after application of sulfur in commercial insecticides.

The yellow-green character has been transferred to several varietal backgrounds and the resulting yellow-green plants were small, slow-growing, and usually had weak main stems. The growth rate of yellow-green plants is one third to one half that of normal plants at night temperatures above 65°F and day temperatures below 100°F. The growth rate is two thirds to five sixths that of normal when night temperatures are above 80°F and day temperatures above 100°F. At night temperatures below 65°F regardless of day temperatures, the growth rate of yellow-green plants is very small in comparison to that of normal green plants.

GENETICS OF YELLOW-GREEN

Plants of a progeny (1171), derived from an original yellow-green plant, were crossed with genetic stocks and commercial varieties of cotton. The F_1 plants were either self-pollinated or backcrossed with yellow-green testers. Two types of segregation ratios were recorded; i.e., 3 green to 1 yellow-green in F_2 and 1 green to 1

TABLE 1

Varieties and normal genetic stocks crossed with yellow-green to obtain segregation in F_2 and backcross progenies

Segregation at one locus, F_2 ratio 3 green to 1 yellow, BC ratio 1 green to 1 yellow				Segregation at two loci, F_2 ratio 15 green to 1 yellow, BC ratio 3 green to 1 yellow			
		Green	Yellow			Green	Yellow
G. hirsutum				*G. hirsutum*			
Coker 100 inbred 2-3-1-7	BC	9	7	Stoneville 2B and	BC	30	9
Coker 100 W	F_2	71	19	Z106, its doubled haploid	F_2	1965	124
Acala 28 or 33	BC	23	26	Triumph	BC	15	3
Acala 44	F_2	208	73	Rogers Acala having D_5 petal spot	F_2	20	0
Rogers Acala	F_2	15	2	Stock 1188 (Rhyne Stoneville x Dwarf Red x Cook 307-6)	BC	18	2
Tri-species line 108	BC	3	3				
Stock 1188-1	BC	20	21				
Stock 1193 (De Ridder Red Leaf x Hopi x S4025)	BC	104	101	*G. barbadense*			
				T 169*	F_2	46	4
				St. Vincent Superfine	BC	22	7
S4025 = SL 7-9	BC	41	40				
S4035†	BC	59	55				
S5082†	BC	10	9	(*G. arboreum* x *G. thurberi*)			
Texas Big Boll	BC	40	38	$2(A_2D_1)$	BC	96	35

* Courtesy of MR. D. M. SIMPSON, University of Tenn., Knoxville.

† Courtesy of DR. S. G. STEPHENS, Department of Genetics, N. C. State College, Raleigh.

yellow-green in backcrosses, or 15 green to 1 yellow-green in F_2 and 3 green to 1 yellow-green in backcrosses. Varieties used to obtain these ratios are tabulated in table 1.

Plants of the 1171 yellow-green tester were crossed with recessive virescent-yellow carried by stocks S4035, S5082, and Texas Big Boll. The F_1 plants were green and the F_2 progenies gave approximately equal numbers of green and yellow plants, suggesting a 1:1 ratio indicative of pseudoalleles, or a 9:7 ratio due to independent loci. The possibility of pseudoallelism was excluded when the F_1 (yellow-green x virescent-yellow) was outcrossed to normal green Z106 and the resulting outcross plants were self-pollinated. (All families resulting from this outcross should segregate for yellow plants if yellow-green and virescent yellow were allelic). However one fourth of the outcross families failed to produce any yellow plants, which indicated independent loci (table 2).

The results in table 2 show that 5 of 24 families produced only green seedlings in F_2. In addition 8 families segregated for virescent-yellow and fit the ratio, 3 green to

TABLE 2

Segregation in families derived by self-pollination of plants from the outcross (yellow-green x virescent-yellow) x normal green

Families			Segregation within families				
Observed	Expected		Green plants	Yellow plants	Ratio	Chi square‡	P
	Allelism	Independence					
Segregating green and virescent-yellow							
8	12	6	1313 (1312.5)	437 (437.5)	3:1 obs 3:1 exp	0.01 1 df 20.59 7 df Het.	0.90 0.01*
Segregating green and yellow-green							
9	12	6	1965 (1958.4)	124 (130.6)	15:1 obs 15:1 exp	0.35 1 df 8.01 8 df het.	0.50 0.30
Segregating green, virescent, yellow-green							
2	0	6	233 95†	82 90	45:19 obs 9:7 obs	1.88 1 df 1 family 1.98 1 df 1 family	0.20 0.10
Not segregating							
5	0	6	995 (995)	0 (0)	995:0 obs 995:0 exp	—	
24	24	24	—	—			

* The test indicated these families were heterogeneous.

† Green plants lost in this family at first cultivation.

‡ Chi Square calculated on total of all families segregating for a gene, unless stated otherwise, hence 1 df for pooled families, n-1 for Heterogeneity among families.

1 virescent-yellow, expected for this known locus in cotton. These 8 families were heterogeneous according to the Chi Square test for heterogeneity reflecting the unfavorable conditions in a desert environment. Similarly for yellow-green, 9 of the 24 families segregated 15 green to 1 yellow, the ratio inherent to F_2 between Z106 and yellow-green. Two other families segregated for both types of yellow plants. One family of these, however, had an excess of yellow plants which probably resulted from an observed accidental removal of strong green plants in this family at the first cultivation by an improperly adjusted rotary harrow.

It may be noted that the 17 families which segregated for either yellow-green or virescent-yellow were parental classes and the remaining 7 families were recombination classes. This ratio of 17 parental to 7 recombination might be attributed to sampling (P = .04) but it could also represent a weak linkage between yellow-green and virescent-yellow of circa 29%. The writer has limited information supporting linkage between the two loci but as yet confirmation is lacking on a large population.

The green F_1, yellow-green x virescent yellow ($yg_2\ yg_1V/Yg_2\ yg_1v$), was backcrossed also to each yellow tester stock. The backcross to virescent yellow ($Yg_2Yg_2\ yg_1yg_1\ vv$) segregated in the ratio of one green to one virescent yellow plant attributed to segregation at the *V* locus. Self-pollinated virescent-yellow plants from this backcross did not segregate phenotypically. The backcross to yellow-green ($yg_2yg_2\ yg_1yg_1\ VV$) segregated in the ratio of one green to one yellow-green plant indicating difference of alleles at the yg_2 locus. One half of the self-pollinated yellow-green plants of that backcross also segregated for the *V* locus in a ratio of 3 yellow-green to one virescent yellow plant. This was interpreted to mean that the multiple recessive genotype has the phenotype of virescent yellow. It was also observed that the multiple recessive plants in such progenies were much more vigorous than sister yellow-green plants. Because of the vigor of the multiple recessives agronomic tester lines have been developed which thus utilize the desirable genetic properties of each locus.

LINKAGE RELATIONSHIPS OF YELLOW-GREEN

The yellow-green 1171 tester was crossed with available multiple dominant and recessive marker stocks and the F_1's backcrossed with yellow-green. Such preliminary tests indicated yellow-green to be independent of the D subgenome markers, okra leaf shape, crinkle, and red plant body. Additional tests showed the independence of yellow-green and the A subgenome marker, yellow-pollen, and also of yellow-green and dominant naked seed which as yet has not been assigned to a genome. (The genome location of the leaf shape and pollen color markers has been established by RHYNE, unpublished). The relationship to yellow petal, brown lint, dwarf red, and petal spot, in these tests could be attributed to either linkage or to poor survival in the recombination classes. In order to clarify some of these ambiguities a yellow-green tester was crossed with two multiple dominant marker stocks and the backcross and F_2 progenies grown. These data are shown in table 3 where it is evident that yellow-green is independent of yellow petal, but certainly is linked with petal spot.

The relationship of brown lint and yellow-green appears to be that of weak linkage. Ordinarily petal spot and brown lint show independence in linkage tests but were peculiarly and tightly linked when the ghost allele of *G. anomalum* causing a large

TABLE 3

Recombination of three dominant genes with yellow-green in F_2 and backcross progenies, coupling phase, one locus segregating of two controlling yellow-green

Character comparison	Phenotypes				(Yellow-green unscored)	Recombination estimate
	Normal		Yellow-green			
	++	−+	+−	−	?−	
Petal spot and yellow-green backcross progenies	Obs Exp.	Obs Exp.	Obs Exp.	Obs Exp.		
4025F_1 x 1171 (1952)	29(20.2)	12(20.2)	4(20.2)	11(20.2)	25	28.6%
1193F_1 x 1171 (1953)	34(20.8)	6(20.8)	4(20.8)	33(20.8)	6*	12.1%
1171 x 1193F_1 (1953)	52(30.5)	12(30.5)	12(30.5)	42(30.5)	4*	19.7%
F_2 Progenies						
4025 (1952)	74(57.2)	14(19.2)	1(19.2)	2(6.4)	11	†
4025 (1953)	14(13.2)	2(4.2)	1(4.2)	3(1.4)	3	—
4025 (1953)	‡	‡	25(66.0)	63(22.0)	26	15.8%
1193 (1953)	86(74.6)	13(24.6)	4(24.6)	15(7.4)	11	13.0%
Brown lint and yellow-green F_2 progenies						
4025 (1952)	†	†	†	†		
4025 (1953)	12(13.2)	4(4.2)	0(4.2)	3(1.4)	4	—
4025 (1953)	‡	‡	48(57.8)	29(19.2)	102	39.8%
Yellow petal and yellow-green backcross progenies						
1193F_1 x 1171 (1953)	16(20.8)	24(20.8)	13(20.8)	23(20.8)	7*	Independ.
1171 x 1193F_1 (1953)	30(30.5)	34(30.5)	26(30.5)	27(30.5)	5*	Independ.
F_2 Progenies						
1193 (1953)	76(74.6)	23(24.6)	16(24.6)	3(8.2)	11	Independ.

* The yellow-green plants unscored do not agree in 1193 backcross populations because petal spot was scoreable at a time of day when petal color could not be critically evaluated.

† These populations were affected by Texas Root rot and most plants killed before capsules matured.

‡ Most normal plants removed to permit flowering of yellow-green plants.

petal spot replaced the smaller petal spot of multiple stock S4025 (RHYNE 1951 and subsequent unpublished data). The data in table 3 will be considered to indicate that petal spot, one locus of yellow-green (Yg_2), and brown lint are members of a common linkage group.

The linkage of one locus of yellow-green with the petal spot locus in those genetic stocks having normal alleles at but one locus is supported by a similar linkage in other allotetraploid species having normal alleles at the two yellow-green loci. In *G. barbadense* almost all varieties have a dominant allele determining petal spot. In a few varieties an allele dominant to petal spot exists, causing red anthocyanin pigmentation on the leaves, stems, and leaf veins. Red T169 having this dominant gene for red plant coloration was crossed to a yellow-green tester and the F_1 self-pollinated and backcrossed to the yellow-green tester. The F_2 segregated in a 15 normal to 1 yellow-green fashion (see table 1) and did not segregate for chlorophyll deficiency as often as is the situation in Upland-*G. barbadense* F_2. The backcross progeny were limited, as can be observed in table 4, since it was difficult to get flowers simultaneously on the species hybrid and the yellow-green tester under field conditions. There was an absence of plants in the recombination class, red and yellow-green, which was consistent with the previous intra-Upland experiments indicating linkage between the petal spot locus and one (Yg_2) of the yellow-green loci. Attempts to get larger

TABLE 4

Segregation in backcross progenies of interspecific (red G. barbadense *and red amphidiploid 2(A_2D_1) x yellow-green* G. hirsutum *tester 1171) to 1171*

Back cross progeny	Phenotypes			
	Normal		Yellow-green	
	Red plant body	Non-red plant	Red plant body	Non-red plant
		Obs Exp		Obs Exp
G. barbadense (T169 x 1171) x 1171				
Seedling stage		42(40.5)		12(13.5)
Mature plant	28(19.86)	14(19.86)	0(6.62)	11(6.62)
Exp. Independence	3/8	3/8	1/8	1/8
Amphidiploid 2(A_2D_1) (2 A_2D_1 x 1171) x 1171				
Seedling stage		96(98.3)		35(32.7)
Mature plant	51(39.0)	31(39.0)	2(13.0)	20(13.0)
Exp. Independence	3/8	3/8	1/8	1/8
Estimated recombination (yellow-green phenotype) red and yellow-green loci			2/22 or 9.0%	

populations for estimating recombination of petal spot and yellow-green were negated when plants of backcrosses, yellow-green x typical barbadense varieties backcrossed to yellow-green, exhibited the usual aberrant behavior (fade out in petal spot) in this interspecific cross (HARLAND 1939). There is probably little doubt that the *G. barbadense* varieties used in this study had normal alleles for yellow-green at two loci, and that one of these was linked with petal spot locus.

Additional evidence of linkage between the petal spot locus and Yg_2 of the two yellow-green loci was obtained by using newly synthesized amphidiploid 2(A_2D_1), *G. arboreum* L. x *G. thurberi* Tod., having a dominant red anthocyanin marker of A_2 and the normal green (reduced anthocyanin level) of D_1. This amphidiploid exhibiting pale red stems, leaves, and leaf veins, and associated red petal margin was hybridized with a yellow-green tester and the hybrid subsequently used in backcrosses with yellow-green. Sterility and seeding lethality diminished the chance of a large progeny but fortunately these interspecific complications were not particularly localized to the phenotypes in the present study. Reduced recombinations (see table 4) were particularly associated with the phenotypic class, red petal margin and yellow-green. The estimate of recombination of red plant-red margin and yg_2 of the yellow-green loci was 2 of 22 plants having yellow-green, or 9%. An estimate of 9% linkage between anthocyanin and lethal yellow seedling found by YU (1939) in intra-*G. arboreum* crosses corresponds to the estimate herein of 9% between the A_2, *G. arboreum*, dominant anthocyanin marker with its normal and linked allele (Yg_2) for yellow-green, and the A subgenome of Upland, recessive anthocyanin and linked recessive yellow-green locus (yg_2). The large number of normal plants (for yellow-green) not having red anthocyanin pigment was obviously similar to normal plants having low anthocyanin pigmentation, obtained in independent genetic test

crosses establishing an allelism of the low anthocyanin factor of D_1 with the common R_1 red plant factor of Upland cotton. This similarity suggests a possibility that the second locus of yellow-green (Yg_1) is associated with the D_1 allele for low anthocyanin, hence the 31 plants (see table 4) having green leaves stemming from a low anthocyanin but normal for yellow-green. If so, the yg_1 locus of yellow-green could therefore be linked with R_1 in intra-Upland varietal crosses.

Several red plant (R_1) tester stocks involving different varietal ancestries were crossed with yellow-green and the hybrids backcrossed to yellow-green. All of these had normal alleles at but one locus (see table 1, also table 3, stocks 1193 and 4025) and this locus was independent of the R_1 locus. In the absence of a cluster and yellow-green tester a stock having red plant and cluster, R_1-cl_1, was crossed to 1171, r_1-Cl_1, normal for cluster habit. The red, non-clustered, normal (for yellow-green) F_1 was crossed with 1171 and the progeny self-pollinated. Because the linkage between R_1 and cl_1 is tight, 5–18% having been reported by various workers, homozygous red plants would be, in general, also cluster, and homozygous red and yellow-green plants should also be cluster. As expected some of the homozygous red plants had cluster but homozygous red, yellow-green plants were normal. Such an absence of cluster in homozygous red, yellow-green plants might be attributed to misclassification, had this abnormality been confined only to red, yellow-green plants. In addition yellow-green progeny of the backcross of multiple recessive tester S5082 (and S4035) x 1171 to 1171 yellow-green failed to segregate for cluster although other recessives from S5082 were recovered, namely virescent-yellow, crinkle, and leaf shape. Extensive progenies were grown, being utilized however for agronomic experiments of McGREGOR, *et al.* (1954) but no yellow-green cluster plants were observed. No experimental hypothesis has been formulated to account for the anomalous absence of cluster plants although a reinvestigation is planned using additional yellow-green stocks and cluster testers. Tentatively it appears that an allele of Cl_2, having a function different than Cl_2 in *G. hirsutum* normally has, may be localized in the yellow-green 1171 stock. Relationship of yellow-green and Cl_2 will be considered associated in a paragraph in the Discussion.

DISCUSSION

The genetic information from F_2 and backcross progenies suggest that yellow-green is a recessive differing from normal green cotton varieties at either one or two loci (table 1). In some of these stocks the only locus having normal alleles is linked with the petal spot locus (table 3). In other stocks normal alleles are at two loci, one locus (Yg_2) is linked with petal spot which is on the A subgenome HARLAND (1935) and the other is, perhaps, that of the D subgenome (table 4). The stocks having dominant, common red leaf (D subgenome HARLAND and ATTECK 1941) tested so far have no normal allele for yellow-green linked with the red leaf locus, but have normal alleles at the locus on the A subgenome which is linked with petal spot. These data are suggestive that diploidization has occurred in *G. hirsutum* and that eventually more varieties might be found having (1) normal alleles at two loci for yellow-green as Stoneville 2B has, (2) only normal alleles at one locus, that of the A subgenome as S4025 has, and (3) perhaps normal alleles at but one locus and that on the D sub-

genome. In contrast to *G. hirsutum* raw allotetraploid *G. arboreum* x *G. thurberi*, $2(A_2D_1)$, has normal genes at two loci, presumably one on each of its genomes. Similarly varieties of *G. barbadense* tested have normal genes at two yellow-green loci, perhaps one on each subgenome.

The inheritances of yellow-green and chlorophyll deficiency as summarized by HARLAND (1939) appear to be similar. The F_2 of hybrids between *G. hirsutum* and *G. barbadense* segregate in the ratio of 15 normal to 1 affected plant. Either of these species may be recessive at one of two complementary loci determining chlorophyll deficiency, although some varieties of *G. hirsutum* have, however, normal alleles at both loci (so that these therefore do not segregate for. chlorophyll deficiency, a complementary duplicate recessive, i.e. F_2 of crosses with *G. barbadense*). HARLAND described the chlorophyll deficients on *G. hirsutum* varietal background as seedlings of uniform yellow color yet eventually lethal. This description of *G. hirsutum* chlorophyll deficiency and yellow-green, as described previously, are similar, but yellow-green seedlings survive. HARLAND (1939) lists the complementary chlorophyll deficiency loci but did not test their independence of the petal spot R_2 and plant body color R_1 loci, whereas one of the yellow-green loci is linked to the petal spot locus, and the second is, perhaps, linked with red plant body in appropriate varieties and species. (DR. MARGARET MENZEL, Department of Agronomy, Texas A and M, College Station, in a letter communication indicated she obtained chlorophyll deficients which were sometimes viable from F_2 of *G. hirsutum* x *G. barbadense* and crossed these with yellow-green stocks donated by the writer. Her F_1 plants were green and hence yellow-green and chlorophyll deficiency, both duplicate recessives, probably involve different loci.)

The origin of yellow-green might be considered as a possible mutation of a normal allele to recessive at a locus on the A subgenome in an F_2 plant of the hybrid between Coker 100 and Acala W29. A second interpretation seems plausible yet some of the evidence supporting this explanation is contradictory. Accordingly, and extending the data reported herein on an admittedly small number of varieties representative of current and past varieties of Upland cotton, varieties of *G. hirsutum* of unlike ancestry might differ in the number and location of loci determining yellow-green and when intercrossed segregate for yellow-green. Coker 100 and Acala W29, the parental varieties of yellow-green, are derived from unrelated sources in *G. hirsutum* and have only recently been intercrossed. Possibly each of the parental types had normal alleles at different subgenome locations from which yellow-green appeared as a rare segregant in the F_3. There are, however, numerous strains of Coker 100, and possibly as many of the Acala W29, so that attempts to obtain segregation of yellow-green in F_2 or F_3 between these dissimilar populations might be without success. Only MR. P. H. KIME of breeders having extensive experience with F_2 between these two dissimilar varieties, found yellow-green. Correspondence with breeders from North Carolina to California indicated no yellow seedlings in F_2 or F_3 between Acala W29 and its derivatives when crossed with Coker 100 strains. Nevertheless DR. S. G. STEPHENS (Dept. of Genetics, N. C. State College, Raleigh) and MR. SMITH WORLEY (Dept. of Plant Breeding, University of Arizona, Tucson) have seen yellow-green segregants (or segregants of similar phenotype) in F_2 involving widely dissimilar parental varieties.

The low recombination value thus indicating linkage between one of the yellow-green loci and petal spot placed this yellow-green locus on the A subgenome of the allotetraploid cottons, since HARLAND (1935) established the allelism of petal spot of *G. hirsutum* and petal spot of *G. arboreum*, its A genome diploid relative. An analogous linkage exists within *G. arboreum* varieties where the anthocyanin locus is linked with a lethal yellow seedling (YU 1939) and it could represent the linkage of the same two loci within *G. hirsutum* which, however, possess alleles of different potency. That locus of yellow-green which is linked with petal spot within Upland cotton may be designated Yg_2. The linkage of Yg_2 and petal spot permits the possibility of developing three (or more) point linkage tests in allotetraploid cotton using petal spot, short branch (KEARNEY 1930), and yellow-green.

The A linkage group, R_2-Cl_2-Yg_2, order unknown, is believed to be duplicated on the D subgenome of the allotetraploids as the plant body, cluster linkage group, and might have accordingly the second yellow-green locus. The D linkage group, R_1-Cl_1-(Yg_1), has been used with the A linkage group by SILOW (1946) to show that *G. hirsutum* and *G. barbadense* have chromosome and gene homology with the Asiatic A genome diploids. Also SILOW showed that *G. hirsutum* has gene Cl_2 which blocks *G. barbadense* cl_2 without affecting cl_1 expression within *G. hirsutum*. Possibly an allele of Cl_2 of higher potency accompanies yellow-green and blocked the cluster cl_1 expression in plants homozygous R_1-cl_1 or r_1-cl_1 having yellow-green, as previously reported herein. STEPHENS (1952) suggests that the A and D linkage groups are duplicated but each has divergent genetic functions of the duplicated genes. Either recessive cl_1 or cl_2 within their respective *G. hirsutum* and *G. barbadense* mutant backgrounds effect a shortening of the fruiting branches without being blocked by a concomitant dominant Cl_2 or Cl_1 generally conceded to be present. Tentatively any proof of the assumption of a Cl_2 gene, possessing greater potency than the usual Cl_2 of Upland, which blocks cl_1 in yellow-green plants, requires reinvestigation of the anomaly using yellow-green in appropriate linkages in stocks as yet unavailable.

MENZEL and BROWN (1952) stress the need of genetic evidence to supplement the cytological data on observed low metaphase chiasmata frequency in Gossypium. Double (and multiple) crossover frequencies are especially needed, but as these workers state three or more point linkage groups are not available in Gossypium (except in *G. arboreum*). Genetic evidence might be developed which relates to cytological information on the basis of the data reported herein, and from unpublished data obtained from test crosses involving both of the allotetraploids and diploids of the A and D genomes. The A subgenome petal spot-short branch linkage group of *G. hirsutum* and *G. barbadense* contains the following genes: R_2-Cl_2-Yg_2 (12–20 units from R_2)-K (40 units from Yg_2). Cl_2 and K have probably never been tested, as cl_2 is rare and presumably is now lost. Cl_2 and Yg_2 could be very closely linked and must be evaluated using the A_2 linkage group such as red plant-red petal margin (R_2)-normal (Cl_2)-normal (Yg_2)-white or dirty white (k) in the raw amphidiploid $2(A_2D_1)$ reported in table 4. The Cl_2 of A_2 unlike Cl_2 of *G. hirsutum* blocks cl_1 (unpublished data) and can be evaluated in backcrosses using cluster testers.

The D subgenome linkage group contains red plant (R_1)-cluster (cl_1)-possibly (Yg_1), and probably dirty white (dw) (unpublished data). Both the A and D subgenome linkage groups could be used to estimate crossing over between and within

the allotetraploid species and possibly relate this estimate to cytologically observed metaphase chiasmata germane to the appropriate chromosomes. Similarly crossing over between the A subgenome of the allotetraploids and A genome diploids species, or between allotetraploid D subgenomes and D genome diploids, could be related to the cytological data using stocks marked with appropriate genes.

The yellow-green character might be of use in plant-breeding techniques as the writer has observed distinct heterosis in hybrids between yellow-green testers and commercial varieties, a situation somewhat similar to heterosis in barley reported by GUSTAFSSON, *et al.* (1950) for chlorophyll factors. The writer (MCGREGOR, *et al.*) has also found use for yellow-green testers, because of their recessive nature, in cooperative field experiments designed to evaluate the possibility of using honey-bees and pollen collecting insects to pollinate cotton and to produce seed for hybrid cotton.

SUMMARY

Normal green varieties of Upland cotton have been found to differ from a yellow-green mutant by dominant (normal) alleles at one or two loci. Some varieties having normal alleles at one locus, called Yg_2, were shown to have this locus linked with petal spot (R_2) of the A subgenome. Yg_2 was also indicated to be linked to brown lint thus making available genes at four loci, e.g., R_2-Cl_2-Yg_2-K for estimating double and multiple recombination data possibly relating to cytologically observed low chiasmata frequency in Gossypium. Other varieties having normal alleles at two loci have the second locus, Yg_1, presumably that of the D subgenome and possibly linked with R_1-cl_1-*dw*. Red and cluster stocks (R_1-cl_1) have normal alleles for yellow-green at but one locus, Yg_2, but cl_1 plants were not found in the recovered yellow-green progeny, which was not explainable unless an assumption was made that Cl_2 of yellow-green differed from the normal Cl_2 of *G. hirsutum*. The recessive yellow-green and virescent-yellow characters involve three loci. One of the two yellow-green loci was possibly indicated to be linked with the virescent-yellow locus in the small test cross which established the independent inheritance of yellow-green and virescent-yellow. The phenotype of the triple recessive genotype is that of virescent-yellow. The inheritance of yellow-green possibly represents diploidization in *G. hirsutum* from a double dominant situation, as in raw amphidiploid $2(A_2D_1)$ and allotetraploid *G. barbadense*, at continuous steps having (1) both dominant loci, (2) one dominant on the A subgenome (a single dominant on the D has not as yet been found), (3) and recessives on both genomes.

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A RECONSIDERATION OF THE BROWN-DOMINANT POSITION EFFECT

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BROWN-DOMINANT (*bw*^{D}) has been described by HINTON (1940, 1946) as a mutant at the brown locus (*bw*, 2–104.5) in *Drosophila melanogaster*. It has been interpreted as being inseparable from what SCHULTZ (see HINTON 1942) considered to be a duplication of salivary gland chromosome band 59E1·2, and although the extra material was referred to as heterochromatin by HINTON and GOODSMITH (1950), their cytological description of the mutant was substantially the same as previously reported. However, they indicate that the distal band is the normal one and the proximal band is the one which usually is heterochromatic. HINTON (1942) described *bw*^{D} as not causing variegation, although HINTON and GOODSMITH noted variegation in the heterozygote between two X-ray induced changes of the mutant. These observations resulted in the interpretation of *bw*^{D} as a euchromatic position effect. The failure of SLATIS (1955) to produce any strong euchromatic position effects at the *bw* locus among many due to heterochromatin prompted this investigation of *bw*^{D}.

RESULTS

Cytological observations

The cytological examination of heterozygotes between *bw*^{D} and wild type confirmed HINTON's (1946) description of what appeared to be pairing of the normal band with either of the two bands in the duplication, although the distal band was usually paired and the proximal band only infrequently so. The chromocenter, various euchromatic regions, and chromosome tips, particularly the tip of 2R (which is very close to the brown locus), were attracted to 59E.

Study of the homozygote indicates that this is an erroneous picture. In some slides the region just proximal to band 59E1·2 is distorted or broken in as many as thirty percent of all cells. It is clear in almost every cell that the extra region is much more than the equivalent of 59E1·2. A number of well stretched specimens agree in showing an insertion of three heavy bands just to the left of 59E1·2 and a single light band has been seen on rare occasion as the rightmost band of the insertion. Stress or breakage tends to occur between the extra bands and 59E1·2, and occasionally pairing with heterochromatin will separate these two regions. The inserted material frequently appears to be banded, but it may show a distinct absence of bands in otherwise excellent preparations. The appearance is very much like that of the proximal regions of the long chromosome arms (see HANNAH 1951).

Variegation

The demonstration of variegation in brown locus position effects is greatly aided by combining the position effect with scarlet (*st*) and using the shadow technique of

observation (SLATIS 1955). The homozygote bw^D; *st* is completely white in eye color in all individuals, as is the genotype bw^D/bw; *st*. However, the genotype $bw^D/+$; *st* is always strongly variegated. At emergence from the pupa the spots can only be seen with the shadow technique, although the eye is orange rather than white. Within a few days the spots have darkened to brown on a lightly pigmented background and can be seen without special techniques. The individual spots rarely exceed a single facet in size, although about one percent of the flies have a large spot of brown color which may cover any number of facets. Spots of scarlet, which would indicate normal activity at the brown locus, have not been seen. The females are distinctly more pigmented than the males and the pigment is strongest in the front of the eye in both sexes. HINTON (1942) failed to observe any variegation even with the aid of vermilion (*v*). On testing, it was found that *v*; *bw* is light orange in color rather than white (with the *v* allele used in this experiment, and presumably with that used by HINTON) and this obscured the variegation when combined with bw^D. With the shadow technique, *v*; $bw^D/+$ is seen to be variegated. Even with the shadow technique it is almost impossible to observe variegation in $bw^D/+$ flies which have not had the brown colored pigment removed by the use of mutants such as *v* or *st*, or at least slightly diluted by a mutant such as purple.

Crossover data

The *bw* locus is bracketed by plexus (*px*, 2–100.5) and speck (*sp*, 2–107.0), which are excellent genes for the measurement of linkage. Crossovers were measured at 25°C and 29°C for the distances *px-bw* and *bw-sp* in the normal chromosomes and in heterozygotes for bw^D. The *px-sp* distance was measured in flies homozygous for bw^D (the *bw* position cannot be marked phenotypically in bw^D homozygotes). The results are shown in table 1. None of the differences between the two temperatures are significant, so only the 25°C data will be studied, as they are standard and more numerous. At 25°C the *px-bw* distance of 3.87 is close to the standard map distance of 4.0, and the *bw-sp* distance of 2.31 is close to the standard 2.5. The overall distance of 6.18 ± 0.34 is almost exactly one standard error from the expected value, which is not a significant departure. In flies heterozygous for bw^D the *px-bw* distance decreases strongly but the *bw-sp* distance decreases slightly, if at all. The overall decrease (*px-sp*) is significant at the .001 level. The homozygote for the inserted bw^D region

TABLE 1

The effect of temperature and genotype on crossing over in the region of the brown locus

Genotype	°C	Number	Crossover distance		
			px-bw	*bw-sp*	*px-sp* ± S.E.
+/+	25	4982	3.87	2.31	6.18 ± 0.34
+/+	29	1609	3.11	2.55	5.66 ± 0.58
$+/bw^D$	25	4625	2.25	1.99	4.24 ± 0.30
$+/bw^D$	29	2528	2.29	2.69	4.98 ± 0.43
bw^D/bw^D	25	5182	—	—	2.10 ± 0.20
bw^D/bw^D	29	2030	—	—	2.27 ± 0.33

greatly reduces crossing over and the *px-sp* distance is only 2.10 units. This is significantly different at the .001 level from both the normal and the heterozygous arrangements.

Since bw^D might be associated with a lowering of crossing over within the entire genome, or at least within the entire second chromosome, a test was undertaken using other loci. Flies homozygous for bw^D showed a distance of 22.0 ± 1.0 units based on 1727 individuals for the 27.0 units between black (*b*, 2–48.5) and curved (*c*, 2–75.5). Some double crossovers were probably overlooked, which might increase the 22 units to an amount which does not differ significantly from the expected value. The *c-sp* distance is made up of 25.0 units on the standard map from *c* to *px*, and 2.1 units from *px* to *sp* in flies homozygous for bw^D. The same 1727 flies also showed exactly 22.0 ± 1.0 units for this distance. A group of 1344 flies were examined for *b-px* and *px-sp* crossovers in flies homozygous for bw^D. The *px-sp* distance of 2.4 units confirmed previous findings (see table 1). The *b-px* distance of 35.8 units was lower than the randomness expected (53.0 units on the standard map), but double crossovers would account for a large part of this.

Despite the reduction of crossing over in the region of the *bw* locus in heterozygotes for bw^D, there is the possibility that an occasional crossover might separate the allele at the *bw* locus from the inserted material. The results of HINTON and GOODSMITH (1950) suggest that this allele is wild type in function, so with all flies homozygous for *st*, females + bw^D +/*px bw sp*, which are white in eye color, were mated with *px bw sp* males. A total of 20,885 flies failed to show any eye pigment, which would have indicated that a crossover had occurred which separated a wild type *bw* locus allele from the insertion. The cultures were allowed to form a second generation and these were also examined, since half the F_1 females would have been of the same genotype as their mothers (although one quarter of the appropriate crossover type offspring might be difficult to observe because of combination with a bw^D sperm). No crossovers were found among 31,250 F_2 flies.

DISCUSSION

Cytologically, the study of brown-dominant is hampered by the investigation of structurally heterozygous animals. In the heterozygote the inserted material is squeezed into a small space, particularly in the center of the chromosome. As both it and band 59E1·2 are heavily stained, it is frequently difficult to determine which of the two is paired with the single dark band in the normal chromosome. Either an accidental displacement or an optical illusion (but more probably the latter) occasionally gives the impression that the inserted material is paired with the normal band and that the adjacent 59E1·2 band is unpaired. In the case of a small deficiency in this region (unpublished observations) a small number of cells showed a similar optical illusion in which a band whose homologue was deficient paired with the other half of an intact band.

On the other hand, the study of the homozygote clearly shows that the inserted region is not equivalent to band 59E1·2, being much longer and physically distinctive. Also, the insertion produces variegation in certain genotypes. Because of these facts,

the inserted material may reasonably be assumed to be chromocentral heterochromatin, and the *bw*^{D} position effect is no different in its action than any of the many heterochromatic position effects which have already been studied at the brown locus (see SLATIS 1955).

Brown-dominant is the first mutant at this locus which clearly indicates that the variegated position effects are caused by the heterochromatin rather than by the centromere, which is usually associated with it. It is of particular interest in view of the recent study which MCCLINTOCK (1951) has made of the data on white-mottled 5 which had been collected by GRIFFEN and STONE (1940). MCCLINTOCK believes that many of the further rearrangements induced in this mutant must have included a small part of the heterochromatin of chromosome 4. MCCLINTOCK has noticed that in certain cases these translocations were to the end of a chromosome arm and did not produce variegation, but further breakage and translocation nearer to a centromere returned the ability to produce variegation. The observation that *bw*^{D} is related to chromocentral heterochromatin supports MCCLINTOCK'S contention that heterochromatin was associated with many of the translocations despite the fact that it was not cytologically recognizable. However, the *bw*^{D} mutant is remarkably strong since it not only suppresses the wild allele in the same chromosome, but it almost completely suppresses the wild allele in the homologue, and this strength is manifested at the end of a chromosome arm. None of the *R*(+) alleles found by SLATIS (1955) had as great an effect on the wild type allele in the same chromosome and none of the *R*(*bw*) alleles had as great an effect on the wild type allele in the homologue, and in all of these cases the heterochromatin was accompanied by the centromere. MCCLINTOCK'S observation relating variegation to the distance from the centromere is based on only five instances, and one of these is of doubtful significance, so that further work on this problem needs to be done.

It was anticipated that the amount of material which has been inserted into the second chromosome to produce *bw*^{D} might be measured as an increase in the crossover distance in this region. The reduction of crossing over to one third of the normal amount in homozygous *bw*^{D} was contrary to expectation, and has an important bearing on various theories of crossing over. These observations show that crossing over is not a function of the sum of the tendencies to cross over of each small part of a chromosome. Instead, there is probably a complex interaction between the various parts of the chromosome, each of which has an effect which increases or decreases local crossing over. The very small amount of crossing over which is observed in the heterochromatic regions of *Drosophila melanogaster* appears to be an expression of a tendency against crossing over. Although this idea is not new, the evidence supporting the idea is. As we do not know that property of the genes or the chromosomes which governs crossing over, and therefore we do not know what the relationship is between mitotic chromosome length and map distance, it might be argued that the apparently large regions of heterochromatin which have so small a crossover length are really short, but are extended in metaphase by the pull of the adjacent centromere. The evidence presented here that the homozygous insertion of this heterochromatin can cause a shortening of the apparent length of adjacent

euchromatin means that the anti-crossover effect is real. We can more readily accept the chromocentral heterochromatin as being composed of many genes or blocks of genes with this evidence that its short length on the crossover map is due to an anti-crossover tendency. There is a slight possibility that *bw*^{D} influences the whole right arm of chromosome 2, but the small deficit of observed crossovers would signify that any such influence must be very small to the left of plexus, if it occurs at all. Therefore, the remarkable reduction in crossing over is a local effect, presumably due to action by the homozygous mutant on its immediate neighbors.

The theory of crossing over at or near "frontiers" between euchromatin and heterochromatin, which has been propounded by WHITE (1945) would suggest that the insertion of heterochromatin into a euchromatic region should be accompanied by an increase in crossing over. As this is not so, these observations have a detrimental effect on the value of the frontier theory. However, the theory is sufficiently flexible to preclude the possibility that these findings are fatal to it.

The other crossover data which were studied had held the hope that the position of the brown locus should be localized further than the limits of 59D9 and 59E1·2, which were established by SLATIS (1955). As appropriate markers were present, it would have been possible to place the position of brown within the last three bands of 59D or the doublet of 59E with only one crossover. The negative result may be the result of insufficient observation, suppression of crossing over, chromosome structural heterozygosity, too small a distance between *bw* and the insertion, or the presence of an amorphic allele on the *bw*^{D} chromosome. The fact that the effect on crossing over which is measured in heterozygotes for *bw*^{D} is greater to the left of *bw* than to the right is probably not important in this problem.

SUMMARY

A reexamination of brown-dominant has resulted in the interpretation of the cytological picture as an insertion of three dark bands and probably one light band between the last band of 59D and the 59E doublet. The inserted material looks like chromocentral heterochromatin. Variegation is produced by this insertion, but it can only be seen if other pigment has been removed by genes such as scarlet, and when the fly is heterozygous for a wild allele. These two observations are consistent with other reports on heterochromatic position effects at the brown locus. The interpretation of this mutant as a euchromatic position effect can now be disregarded.

Brown-dominant causes only a slight reduction in crossing over when heterozygous with a wild type chromosome, but crossing over in its immediate vicinity is remarkably reduced in the homozygote. There is little or no effect on the remainder of the chromosome arm. The reduction of crossing over indicates that this inserted heterochromatin has a tendency to block crossing over despite the fact that the amount of chromatin present is greater than normal and the chromosomes are structurally homozygous. Therefore, crossing over cannot be interpreted as the sum of the tendencies to recombine of each of the parts of the chromosome. Instead, it must be assumed that each part of the chromosome contributes some characteristic quality to increase or decrease crossing over and the observed crossovers are the result of an interplay of many factors.

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A COMPARATIVE STUDY OF INDUCED MUTATION IN THE OOCYTES AND SPERMATOZOA OF DROSOPHILA MELANOGASTER

II. Deficiencies and Minutes[1]

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IN THE previous paper of this series (Glass 1955) it has been shown that in mature oocytes of *Drosophila melanogaster* translocations are scarcely ever induced by X-ray treatment, whereas inversions are induced, by the same dose and in the same genetic strain, at about one-fourth the frequency with which they are induced in spermatozoa. It consequently appeared probable that the difference between the responses of the mature male and female germ cells to ionizing radiation is not a matter of a different frequency of chromosome breakage, but rather is attributable to a different probability of recombination vs. restitution. This, it was pointed out, might be tested by studying the frequency of chromosomal rearrangements in which the two breaks involved in the rearrangement occurred in very close proximity to each other; for in that case the "non-random factor" responsible for the higher probability of recombination for breaks close together and in the same chromosome might be more nearly equal in the male and female germ cells. Small, two-break rearrangements can either be recovered as deficiencies or as inversions; but, inasmuch as very short inversions are difficult to detect genetically, a comparison of the frequencies of deficiencies in oocytes and spermatozoa was selected as the method of choice.

When the breaks in the chromosome are more than a very short distance—that is, more than a few salivary chromosome bands—apart, the individual breaks are the result of distinct "hits" by the ionizing radiation on the chromosome. But when the breaks are extremely close together, the deficiencies produced increase as the first power of the dose; that is to say, in such instances two chromosome breaks appear to result from a single "hit" (see Muller 1940; Lea 1947, pp. 233–234; Catcheside 1948, p. 308; Kaufmann 1954, p. 668). It is the latter type of deficiency that might be expected to provide the answer to the question posed here. The genetic methods of detection, however, offer no simple way of separating the 2-hit deficiencies from the 1-hit deficiencies.

Initially, an experiment was undertaken to determine the relative frequencies of homozygous-viable mutations and of deficiencies which in both cases involved the loci of certain recessive marker genes in chromosome 2. The largest deficiencies might be readily recognized, because they would involve a simultaneous loss of adjacent marker loci. Smaller deficiencies might be recognized by the lethality of the "mutation" when, by appropriate crosses, it was made homozygous. This of course assumes on the one hand that all point mutations are viable when homozygous, and on the

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lethals, and visibles from treated females to be associated with sectional dislocations that reduced crossing over, whereas 2 out of 12 mutations from treated males were associated with chromosomal aberrations.

In discussing the significance of these rather preliminary results, PATTERSON and MULLER advanced the suggestion that point mutations might be due to changes within a gene that would still leave it to the power to multiply, whereas chromosomal alterations might result from destruction of the gene and loss of its power to multiply, thus bringing about a break in the continuity of the chromosome. From this they reasoned further that either restitution or reattachment to other points might follow breakage; and the process of reattachment might well be subject to different influences than those which affect point mutation. Either the physiological condition of the chromosomes, or the spatial relations between chromosomes tightly packed together as in the sperm head, or loosely dispersed as in other cells, might alter the relative proportion of recombination and restitution. Thus the high frequency of chromosome aberrations found to be associated with mutations induced in spermatozoa might be accounted for, "though the number of 'point mutations' need not be correspondingly more frequent." They wisely refrained from further speculation at the time, but, as we shall see, this suggestion of theirs came very close to actuality.

SHAPIRO and NEUHAUS (1933) obtained 30 translocations from 434 tested spermatozoa (6.91%) following X-ray treatment, but only 1 translocation in 550 X-rayed female germ cells (0.18%). This ratio, of about 35 times as many translocations in the male germ cells as in the female, they found to be about the same as the ratio of translocations induced in mature spermatozoa to those induced in immature male germ cells. OLIVER (1935), on the other hand, reported that one-third to one-half as many translocations involving the X chromosome were induced in treated female germ cells as in similarly treated male germ cells. However, in his abstract no figures were given, and the full data have never been published. If these chromosome aberrations were detected solely by means of their alteration of crossing over frequencies in the X chromosome, as in OLIVER's other studies, it is not clear what proportion of them may have been deficiencies or inversions rather than translocations.

Other workers (HANSON and HEYS 1929; HARRIS 1929; TIMOFÉEFF-RESSOVSKY 1930) reported that a given dose of X-rays produces more mutations in mature spermatozoa than in immature male germ cells. Although it was recognized that this might mean that the latter are much less susceptible to X-rays than those which are mature, TIMOFÉEFF-RESSOVSKY (1934) expressed his opinion that germinal selection was a better explanation. Evidence in support of this was seen in SIDOROFF's finding (1931) that the frequency of autosomal recessive lethals does not diminish in successive broods of the progeny of treated males, although translocations, according to SHAPIRO (1931), do so. The former would not be subject to germinal selection because of their recessiveness, whereas most translocations might be supposed to be eliminated by aneuploid segregation during meiosis.

In Drosophila, fertilization takes place when the oocytes have reached the first meiotic metaphase (see SONNENBLICK 1950). Oogonia and oocytes, like spermatogonia, are immature, i.e., pre-meiotic, germ cells, and would therefore be expected to yield lower frequencies of mutation than spermatozoa. Even in mature oocytes, any

induced aberrations must still pass through meiotic segregation; in other words, they are conceivably subject to germinal selection by means of the elimination of aneuploid combinations. The emphasis on germinal selection as the explanation for the lower frequency of sex-linked lethal mutations and chromosome aberrations in the immature male and all the female germ cells tended in subsequent years to obscure the value of the alternative theory proposed by PATTERSON and MULLER. Thus SCHULTZ (1936), after reviewing the subject, concluded: "On the whole, it appears that the existing data can best be understood in terms of similar rates of mutation in all cells."

Later on, it was demonstrated that dosages measured by different observers under different conditions and with different instruments, or even by the same observer at different times, cannot be reliably equated. Moreover, different strains of the same species may exhibit different mutation rates when exposed to the same dose of X-rays (DUBOVSKIJ 1935; DEMEREC 1938). It is consequently important to note that the comparative method utilizes the analysis of simultaneously treated male and female germ cells of the same genetic strain. In this way variations in the measurement of dosage or of subsidiary conditions of treatment, as well as variations in the sensitivity of different genetic strains, become of little account, since the series to be compared are identical in genotype and were treated identically.

TRANSLOCATIONS

Studies along these lines were made jointly by L. J. STADLER and the author in 1936–40, in order to see whether X-rays produce translocations by simultaneous chromosome breakage and reunion, or whether, on the contrary, chromosome breakage is followed by a reunion of broken ends only after a certain interval has transpired. The method used was to irradiate impregnated females so as to expose the chromosomes in both male and female germ cells to X-rays, and then to make a genetic test of all induced translocations to see whether any of them might involve a reunion between maternal and paternal chromosomes, which of course were in separate germ cells at the time of treatment. The method of detection was the standard genetic test for translocations. Wild type males [in series 12–15, y^2 *v f* males; in series 16, *al Sp b* L^{34}*/Cy*; *Ly Dl Pr/In(3LR)Cx, D*] were mated with virgin *pr cn*; *by*; *ci* ey^R females.[2] In series 1–15 (table 1) the female was X-rayed after insemination; in series 16 (table 1) the males and females were irradiated simultaneously before mating. In all the following experiments, except where otherwise stated, the treated parents were removed from the culture bottles 5 days after the treatment. The progenies were thus composed of single 5-day broods. F_1 males were then mated with *pr cn*; *by*; *ci* ey^R females, and the progeny was examined for the non-occurrence of any of the 8 classes of males and 8 classes of females to be expected on the basis of independent assortment. Whenever a translocation was found to have occurred, both comple-

[2] y^2, yellow-2, 1–0.0; *v*, vermilion, 1–33.0; *f*, forked, 1–56.7. *al*, aristaless, 2–0.0; *Sp*, Sternopleural, 2–22.0; *b*, black, 2–48.5; *pr*, purple, 2–54.5; *cn*, cinnabar, 2–57.5; L^{34}, Lobe-34, 2–72.0; *Cy*, Curly, located in *In(2L)Cy*. *Ly*, Lyra, 3–40.5; *by*, blistery, 3–48.7; *Dl*, Delta, 3–66.2; *Pr*, Prickly, 3–90.0; *In(3LR)Cx*, *D*, inversion involving both arms of chromosome 3, used as suppressor of crossing over. *ci*, cubitus interruptus, 4–0.0++; ey^R, eyeless-Russian, 4–0.2.

TABLE 1

Translocations induced in Drosophila melanogaster spermatozoa and oocytes treated simultaneously

Series	Dose, r	No. gametes tested (per sex)	Trans. in ♂	Trans. in ♀
1	1800	50	5	0
2	1080	49	0	0
3	900	28	0	0
4	1800	28	2	0
5	2700	4	0	0
6	2000	15	2	0
7	3000	24	4	0
8	(not recorded)	38	6	0
9	(not recorded)	141	11	0
10	(not recorded)	59	5	0
11	(not recorded)	65	0	0
12	(not recorded)	12	0 + (1?)	0
13	(not recorded)	30	1 + (1?)	0
14	600	277	6	0
15	2400	95	14	0
16	1500	342	17	0
Total		1257	73 + (2?)	0

mentary types of individuals were tested to determine whether the translocation had occurred in the treated male gamete or the treated female gamete. For example, in the case of a translocation between chromosomes 2 and 3, the commonest type observed, only wild type and purple cinnabar blistery phenotypes would occur (irrespective of sex and the fourth chromosome segregants). Both (a) wild type and (b) purple cinnabar blistery males would then be tested. The wild type males were tested by backcrossing to *pr cn*; *by*; *ci* ey^R females. If the translocation had been induced in the treated male gamete, the wild type males would yield only the two previously found types of offspring; if not, they would yield both (c) purple cinnabar and (d) blistery types as well. It required a somewhat more elaborate procedure to test the purple cinnabar blistery males of the original test progeny. They were crossed to wild type females, and 7 to 10 of their sons were then crossed singly to *pr cn*; *by*; *ci* ey^R virgin females. If a translocation had been induced in the original treated female germ cell, some of these progenies should show only two classes, (a) wild type and (b) purple cinnabar blistery, instead of four classes. In 16 series given various doses (table 1), a total of 73 translocations was induced in 1257 tested progeny, representing equal numbers of treated spermatozoa and mature oocytes. All 73 were found to have been induced in the male germ cells. The experiment therefore failed to answer the question originally posed, but it brought to light the interesting fact that translocations were apparently not inducible in female germ cells.

This finding throws some doubt on the interpretation given by SIDKY (1940) of a remarkable 3,Y translocation found in the progeny of an X-rayed male mated to an untreated attached-X ($\widehat{yy}$; *bw*; *e*; *ey*) female.[3] Among various possible explanations,

[3] $\widehat{yy}$, yellow in attached-X chromosomes; *bw*, brown, 2–104.5; *e*, ebony, 3–70.7; *ey*, eyeless, 4–0.2.

SIDKY came to the conclusion that a translocation between a paternal third chromosome and a maternal Y chromosome furnished the best explanation. In the light of the great rarity of translocations in female germ cells, even when X-rayed, any involvement of the Y chromosome of the $\widehat{XX}Y$ female seems very doubtful. Instead, a translocation between the paternal, X-rayed Y and third chromosomes in a spermatozoon seems more likely. SIDKY felt that this was ruled out by the relative scarcity of non-ebony females in the progeny of the F_1 males, which would presumably in such a case have two Y chromosomes, the paternal one involved in the translocation and the maternal normal Y. He says: "But that would mean that the ring-X-chromosome would segregate freely with either of the Y chromosomes, resulting in a larger number of exceptional—non-ebony—as well as numerous ebony females." Random segregation of the ring-X and the two Y's would yield one-third non-ebony females. The actual number of non-ebony females was not given in SIDKY's paper, and from his ambiguous remark it is not clear how scarce the non-ebony females were, although they apparently did occur. The possible interpretation of a purely paternal translocation is therefore not ruled out, particularly if it can be supposed that the segregation was non-random. Alternatively, one can suppose that in oocytes the Y chromosome is more breakable by X-rays than any other chromosome; or that a break remains open longer, so as to have permitted an exchange between a broken maternal Y chromosome and a broken paternal third chromosome. This hypothesis may readily be tested by X-raying $\widehat{XX}Y$ females and mating them to treated or untreated males.

In further studies male and female Drosophila were irradiated simultaneously in 4 series at a dosage of 2000 r units (60 kvp, 15 ma., about 160 r/min.). In six of these series, a check was made for the occurrence of translocations involving the X-chromosome in treated spermatozoa, in order to have a basis of comparison for the frequency of X-to-autosome translocations in female germ cells. The breeding plan was to irradiate *f B* ♂♂ and then to cross them to *Cy/Pm*; *H/Sb-C* ♀♀.[4] The progenies of the F_1 Bar Curly Stubble females were then examined for failure of free recombination. The inversions present in chromosomes 2 and 3 (*Cy* and *Sb-C*) effectively prevented crossing over in those chromosomes, and the customary reduction of crossing over in translocation chromosomes was relied upon to prevent much recombination between the locus of *B* and any X chromosome break. The method, although not perfect, is seemingly quite efficient, for no borderline cases were found that upon retesting failed to prove to be translocations.

Table 2 gives the results from these experiments, together with the foregoing, and reveals that the frequency of translocations induced in spermatozoa by doses of X-rays averaging about 2000 r is as much as 150 times greater than that in mature oocytes. KANELLIS and RADU (1943) reported obtaining 2 translocations in 1813 oocytes given 4500 r, a frequency of 0.11 percent. This may be compared to the frequency of 0.07 percent obtained in the present series, or the frequency of 0.04 percent if all the series reported in table 2 are combined.

What are the causes of this observed difference in the frequency of induced translocations in male and female germ cells? It might be supposed, first of all, that because

[4] *B*, Bar, 1–57.0; *Pm*, Plum, 2–104.5, inseparable from *In(2LR)Pm*; *Sb-C*, Stubble, 3–58.2, accompanied by *In(3R)C*; *H*, Hairless, 3–69.5.

TABLE 2

Translocations induced by X-rays in spermatozoa and oocytes treated simultaneously

Dose	♂♂			♀♀		
	Gametes tested	Trans-locations	Percentage	Gametes tested	Trans-locations	Percentage
Various doses (600r–3000r)	1257	73	5.81	1257	0	0.00
2000r *f B*	92	3	3.26	198	0	0.0
2000r Ore-R	264	19	7.20	286	0	0.0
	337	26	7.72	392	1	0.26
	437	29	6.64	466	0	0.0
Total	1130	77	6.81	1342	1	0.07
Grand total	2387	150	6.28	2599	1	0.04

the mature oocytes treated in the females are halted in the first metaphase of meiosis, any translocations that were induced might be selectively eliminated during meiosis, or would fail to give viable offspring because of aneuploidy resulting from segregation. Chromosome or chromatid breakage and exchange of an asymmetrical type would result in a dicentric chromosome and an acentric fragment; but any such translocation would be eliminated, whether it occurred in sperm cell or oocyte. The differential probability of transmission is then restricted to symmetrical translocations. A chromosome exchange induced in a sperm cell would be transmitted through mitosis without elimination; a chromatid exchange would presumably be lost in one half of the cases, because of failure of the two chromatids involved to enter the same daughter cell at the first subsequent mitosis. As for female germ cells, it can be calculated that one fourth of the cases of chromosome breakage and reunion (occurring in mature oocytes) would be transmitted; for in Drosophila one half of the metaphase I orientations would result in aneuploid combinations, and one half of the orthoploid gametes do not carry the translocation because it is heterozygous. For chromatid exchanges the reasoning is the same, except that at the second meiotic division there is a further reduction in transmission of the translocation to one fourth. Hence the probability of transmission of a chromatid exchange is $\frac{1}{4} \times \frac{1}{4}$, or $\frac{1}{16}$. Consequently, the probability of transmission of a chromosome exchange is 4 times as great for spermatozoa as for oocytes; and for a chromatid exchange, it is 8 times as great. It therefore seems that the great reduction of translocations in the tested female germ cells must be due either (a) to a different frangibility of the chromosomes in male and female germ cells, or (b) to a different probability that broken chromosomes in male and female germ cells will reunite so as to produce translocations. It seemed possible to test this alternative. If the latter reason were the true one, and if in oocytes the low probability of reunion to yield translocations is due to distance between the chromosomes or to their relative immobility prior to the time at which the broken ends heal, then agents that would stimulate greater movement on the part of the chromosomes in the oocytes ought to produce an increase in the frequency of translocations in the female germ cells.

The influence of supplementary infrared radiation on the induction of translocations by X-rays

The first such agent to be tried was near infrared radiation (GLASS 1949), because of the fact that KAUFMANN, HOLLAENDER, and GAY (1946) had already shown that a treatment with infrared radiation of about 10,000 Å before giving a dose of X-rays greatly increases the frequency of translocations and other chromosome aberrations induced in Drosophila spermatozoa. KAUFMANN's results had been obtained from a cytological analysis of salivary gland chromosomes in the larval offspring of treated males. For our purposes it seemed desirable to stick to the method of genetic detection, so as to keep all our series of data comparable and to permit detection of the numerous translocations with breaks confined to the heterochromatic regions of the chromosomes, such translocations being very difficult, or impossible, to detect by cytological means.

The general method used successfully by KAUFMANN *et al.* (1946), and also by SWANSON (SWANSON and HOLLAENDER 1946; SWANSON 1949; SWANSON and YOST 1951) with Tradescantia, was applied. A commercial infrared lamp (250 watts) was used with a cooling system, a pair of plano-convex condensing lenses, and a glass filter. The cooling system used at the start (series 1–3) was like that used by KAUFMANN *et al.*, and consisted of a flat-sided white glass battery jar filled with a saturated solution of iodine in carbon tetrachloride, 4 cm. in thickness, and cooled by means of a U-shaped glass coil with circulating tap water. A cooling system that proved after some trial to be more convenient and entirely adequate was composed of two units: (a) a glass absorption cell 10 x 10 x 2 cm. (series 4–8) or 21.5 x 14 x 1 cm. (series 9–12) inside measure through which tap water was circulated from a low intake opening to a high outlet; and (b) a copper coil ¼ in. in diameter through which cold tap water circulated and which made 3 loops around the bottom and 3 loops around the top of the shell vial containing the flies. The vial was so placed that the focal point of the infrared beam was in the middle of the volume of air within the vial. However, a sharp focus was avoided, since it was feared that the flies, even though confined to a space 3 cm. high between two corks covered with moistened filter paper, would be able to escape from a sharply focused beam. The beam was therefore regulated to cover the space occupied by the flies ($30 \times \pi(4.5)^2$ mm.) as uniformly as possible. For this purpose the condensing lenses proved to be unnecessary, and in series 9–12 were discarded. A large electric fan was also used to blow cool air over the infrared lamp and the side of the cooling cell exposed directly to the lamp. The temperature inside the shell vial never rose to more than 28°C. during the course of any of the exposures, which lasted up to 11½ hours duration.

In the first eight series with infrared supplementary treatments, the 2 cm. thick glass cell was used as a heat filter. When it was realized that this might be absorbing a significant portion of the infrared at 10,000 Å, the thinner cell was substituted (series 9–12). In this final arrangement the cooling cell, 8 cm. from the infrared lamp, was mounted over an aperture in one side of a wooden box 16 cm. deep. The glass infrared filter, described below, was mounted over a smaller aperture, 6 x 6 cm., on the opposite side of the box, and the cooled vial containing the flies was placed 3 cm. from the filter. Furthermore, when SWANSON's results with Tradescantia indi-

cated that elevated, or even normal, temperatures may nullify the effect of infrared radiation (SWANSON and YOST 1951), the infrared treatments were administered at low ambient temperatures (9°C. for series 10 and 11; room temperature of 18°C. for series 12).

The infrared filter used was a Corning filter 2550, color specification 7–57 (Sextant Red), 3⅛ in. square and 2 mm. thick. This filter is very similar to the one used by KAUFMANN *et al.*, but cuts off more sharply at 27,500 Å and transmits more energy between 7500 and 10,000 Å. It transmits 7% of the radiation at 7500 Å and more than 73.2% at 10,000 Å. One cm. of water cuts off one third of the infrared at 10,000, about two thirds at 12,500, and all at 4,000 Å. The importance of a very thin water filter is therefore obvious. The use of water as a filter in combination with the Corning filter 2550 limited transmission of the radiation chiefly to the wave-lengths between 8000 Å and 13,000 Å, with a maximum at 10,000 Å.

The interval between completion of an infrared pretreatment and commencement of the X-ray treatment was limited to the time required to convey the flies from the place of treatment to the X-ray machine. In no instance was the interval more than 2 minutes, and often it was a matter of seconds. The same applies for the interval between completion of an X-ray treatment and commencement of a supplementary infrared posttreatment.

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Table 3 summarizes the results of the infrared series, when the infrared was given as a supplementary treatment either before or after the treatment with X-rays. No statistically significant difference between the series treated with infrared and the controls treated with X-rays alone was found, although these experiments were repeated twelve times in the course of four years. There may be a slight effect of the infrared posttreatment, contrary to the findings of KAUFMANN and WILSON (1949); for the frequency of translocations induced in spermatozoa by 2000 r units of X-rays averaged just one percent higher with an infrared posttreatment than without; but χ^2 is only 1.33 for two degrees of freedom, and P = .20–.30. That there is some effect of an infrared posttreatment is substantiated by the results for the treatment of the oocytes. Six of the seven translocations so far induced in female germ cells have come from the series given an infrared posttreatment. Although such a result might also be obtained by chance alone, the concurrence of the results in the males and females does indicate a positive, though slight, effect of the infrared radiation in increasing the frequency of translocations.

The reason for the marked conflict between these results and those reported by KAUFMANN *et al.* (1946, 1949) must be considered. Every precaution was taken in the present studies to ensure that the flies received an adequate dose of infrared radiation. In Series No. 6 the time of infrared treatment was extended to 11½ hours, although KAUFMANN had reported that a maximum effect was obtained after no more than two hours. Exposure of the flies to infrared was given with the same apparatus and set-up that was used on the same day for treating Tradescantia buds with the usual

TABLE 3

The effect of supplementary infrared radiation on the induction of translocations by X-rays

Series	Dose	X-rays only			Infrared pretreatment			Infrared posttreatment		
		N.	T.	%	N.	T.	%	N.	T.	%
A. Spermatozoa										
1, 5	2574r	114	5	4.4				114	7	6.14
2, 5	2480r	232	15	6.5				232	21	9.05
3	2000r	92	3	3.26	95	7	7.38			
4	2009r	260	11	4.26	268	11	4.1	237	19	8.02
6	1990r	156	10	6.4				125	4	3.2
7	1991r	166	10	6.03				156	9	5.77
8	2000r	199	9	4.5				237	21	8.86
9	1950r	264	19	7.2				257	14	5.5
11	1978r	337	26	7.7	355	27	7.6	337	29	8.6
Total	2000r	1474	88	5.97	718	45	6.27	1349	96	7.12
Total	2500r	346	20	5.78				346	28	8.1
12	4000r	79	9	11.4				142	15	10.6
B. Oocytes										
1	2574r							169	1	
2	2480r							243	0	
3	2000r							454	0	
9	1950r	286	0					275	4	1.5
10	1978r	392	1		389	0		393	1	
Total	2000r	678	1	0.15	389	0		1122	5	0.54
	2500r							412	1	0.24
								1534	6	0.45

N, gametes tested. T, translocations found.

Duration of infrared treatment: series 1, 4 hr.; series 2–4, 6 hr.; series 6, 11½ hr.; series 7–9, 5 hr.; series 10, 3¼ hr.; series 11, 3 hr.; series 12, 3 hr. pretreatment + 3 hr. posttreatment.

positive results. After SWANSON and YOST (1951) had found that a high circumambient temperature could nullify the effects of the infrared radiation, Series Nos. 10 and 11 were run at 9°C. and Series 12 at 18°C., with the same results as before, namely, no difference when the infrared treatment was given before the X-ray dose, and about a 1% increase when the infrared was given afterwards. The divergence in results being so complete, additional work must be done to clarify the matter. It may be suggested, however, that perhaps the frequency of translocations detectable by salivary chromosome analysis is much higher than that detectable by genetic analysis. In other words, most of the aberrations detectable in the salivary gland chromosomes are perhaps of a non-viable type that would seem to be eliminated between the third instar of larval life and the formation of germ cells in the adult male. If this were so, the discrepancy in the reported effects of infrared supplementary treatments in

Drosophila might be explainable by postulating that the infrared particularly increases the frequency of non-viable chromosome aberrations, but that it does not greatly alter the frequency of viable ones.

Frequency with which particular chromosomes are involved in translocations

Table 4 gives the distribution of the translocations according to the chromosomes involved. The small fourth chromosome was involved only 8 percent of the time. Chromosomes 2 and 3 were involved almost exactly equally often, about 80 to 85 percent of the time. The X chromosome was involved rather less than half as frequently as chromosomes 2 or 3; and the Y chromosome was involved somewhat less frequently than the X, and about one third as often as either large autosome. These frequencies may be compared with those found for breakage and recombination in the salivary gland chromosomes. They agree very closely with those which may be calculated from the data of BAUER, DEMEREC, and KAUFMANN (1938, table 2) by halving the frequency of breakage per chromosome in their data, and comparing the results with the frequencies of involvement of particular chromosomes given here in table 4. (This comparison assumes that the frequencies of triple and higher multiple-break rearrangements are so low that they may safely be neglected.) In comparison to the findings of BAUER *et al.*, the present data reveal a slightly higher frequency for involvement of chromosomes 2 and 3 (+2% and +3.6%, respectively), a rather lower frequency for the X and Y chromosomes (−2.3% and −1.35%, respectively), and a higher frequency for chromosome 4 (+2.5%). The present data agree with those based on the salivary gland chromosomes in indicating that chromosome 2 is slightly more often involved than chromosome 3, and the X about 3.0% more than often the

TABLE 4

Distribution of translocations according to chromosomes involved

	Total	Y,2	X,2	Y,3	X,3	Y,4	X,4	Y,2,3	X,2,3	2,3	2,4	3,4	2,3,4
Induced in spermatozoa													
Tests for Y,2,3,4	75	9		11		0		1		46	3	2	1
Tests for Y,2,3	284	41		43				6		194			
Tests for X,2,3	59		13		5				2	39			
Induced in oocytes													
Tests for X,2,3	10		1		4					5			

Percentage involving a particular chromosome

Y	X	2	3	4
21/75 (28.0%)		60/75 (80.0%)	61/75 (81.3%)	6/75 (8.0%)
90/284 (31.7%)		241/284 (85.0%)	243/284 (85.6%)	
	25/69 (36.2%)	60/69 (87.0%)	55/69 (79.7%)	
111/359 (30.9%)	25/69 (36.2%)	361/428 (84.4%)	359/428 (83.8%)	6/75 (8.0%)

Y chromosome. The data of BAUER (1939, table 25) are not in quite so good agreement, for he lists translocations that total 50 X-2, 55 X-3, and 102 2–3 exchanges. This result gives a ratio of involvement of 50.7%, 73.4%, and 75.8% for X, 2, and 3 respectively, in comparison to 36.2%, 87.0%, and 79.7% in the present tests (last row above totals, table 4). Not only do BAUER's data in the 1939 papers give a higher frequency of involvement of the X chromosome, but chromosome 3 also slightly outranks chromosome 2. It is perhaps worth remarking that in the present data chromosome 3 was in fact more often involved than chromosome 2 in the more extensive series of tests of Y-bearing spermatozoa, whereas chromosome 2 outranked chromosome 3 in the tests of X-bearing sperms and of oocytes. This relationship agrees with BAUER's results, but no substantiation can be found in the data of BAUER, DEMEREC, and KAUFMANN. Probably none of these minor differences are therefore real.

Frequency of translocations in gametes of different ages

In those series in which broods were tested separately (series 9–11), it was found that the frequency of translocations induced by X-rays in spermatozoa is lower in the first four-day brood and the next three-day brood than in the final four-day brood (table 5). These data show that for X-rays, just as for nitrogen mustard (KAUFMANN, GAY, and ROTHBERG 1949), there is an increase in the frequency of induced translocations in spermatozoa utilized from the 8th or 9th day after treatment through the 11th or 12th day. AUERBACH (1951) has attributed this rise to the maturation of germ cells that were immature at the time of treatment, for increasing the duration of storage of mature, treated spermatozoa does not result in any increase in the frequency of mutations. On the other hand, these data do not agree with those of CATSCH and RADU (1943), who found that there was at first a very high rate in the most mature spermatozoa, namely, those used on the first day after treatment; next, a drop to a level at about 50 percent of the initial frequency, the new level being maintained through the 13th day; and finally, a marked reduction to very low levels. If the present data are valid, it follows that translocations, like dominant lethals, sex-linked recessive lethals, and autosomal recessive lethals (AUERBACH 1951; BONNIER and LÜNING 1950; LÜNING 1952 a, b), show an increase in sensitivity in male germ cells beginning about six or seven days following treatment either with X-rays or with one of the mustards, although only in the case of translocations has this been demonstrated for both the ionizing radiation (present experiments) and the chemical mutagen (KAUFMANN, GAY, and ROTHBERG 1949).

As for the female germ cells, it is noteworthy that all translocations obtained to

TABLE 5

Frequency of translocations in male and female germ cells used at various intervals after treatment

	♂♂			♀♀		
	Gametes tested	Translocations	Percentage	Gametes tested	Translocations	Percentage
Brood A (days 1–4)	540	34	6.3	773	6	0.78
Brood B (days 5–7)	529	28	5.3	777	0	0.0
Brood C (days 8–11)	481	53	11.0	184	0	0.0

date were produced in the most mature oocytes. No data of this sort seem to have been previously reported for translocations in Drosophila, although a reduction in the frequency of mutations induced in oogonia (data from eggs laid more than seven days after treatment) as contrasted with the frequency in oocytes (data from eggs laid within the first seven days following treatment) has been reported in abstracts by MULLER, VALENCIA, and VALENCIA (1950) for sex-linked visible mutants, and by GALL (1950) for sex-linked lethals. GLASS (unpub.) has shown that a similar difference applies also to dominant Minutes, other autosomal dominants, autosomal recessives and deficiencies, and sex-linked semilethals, and has confirmed the lower rate for the sex-linked lethals and visibles. These results are entirely consistent with the reported relations in Sciara and Habrobracon. In the former (cf. BOZEMAN and METZ 1949), sensitivity, as measured by the frequency of chromosome aberrations detected in the salivary gland nuclei, is zero during the long germinal vesicle stage before the prophase of the first meiotic division begins. It then rises through prophase to metaphase, and reaches a peak in mid-anaphase. Meiosis is halted at late anaphase pending fertilization. In Habrobracon, WHITING (1945) found that metaphase I was far more sensitive to X-rays than was prophase I, as gauged by egg hatchability and apparently also by chromosome fragmentation.

INVERSIONS

The data accumulated in regard to the relative frequencies of translocations in female germ cells made it of interest to test also the relative frequencies of inversions. Like the commonest class of translocations, most inversions involve two distinct breaks, but with the difference that in the inversions the two breaks fall within the limits of the same chromosome ("intrachromosomal exchanges"), whereas in translocations they are in separate chromosomes ("interchromosomal exchanges"). It is well established, for a variety of plant and animal species, that "the proportion of exchanges in which both breaks are in one chromosome, rather than in separate chromosomes, is higher than would be expected on the basis of random union between breakage ends" (CATCHESIDE 1948, p. 284). CATCHESIDE himself (1938), HELFER (1941), and KOLLER and AHMED (1942) have demonstrated this to be true for Drosophila spermatozoa. It thus becomes of interest to determine whether the same ratio between translocations and inversions, found under given conditions in the spermatozoa, also holds for oocytes under those same conditions. Said differently, is the ratio between the frequencies of translocations induced in oocytes and spermatozoa treated with the same dose of X-rays the same as the ratio between the frequencies of inversions in male and female germ cells treated simultaneously? The answer might be expected to throw light on the question whether the differences in response to ionizing radiation on the part of the oocytes and spermatozoa are due to differences in the frequency of chromosome breakage, or rather to differences in the probability of recombination between breaks. The latter in turn might be either a result of differences in the rate or probability of "healing," or else of differences in the amount of chromosome movement between the times of breakage and recombination.

Unfortunately, inversions require far more labor to detect genetically than do translocations; and, for the reasons already stated, a comparison with cytological analyses of salivary gland chromosomes scarcely appears to be valid. Four series of

TABLE 6

Comparative frequencies of inversions induced in X-rayed spermatozoa and oocytes of D. melanogaster

Dose	♂♂			♀♀		
	Gametes tested	Inversions	Percentage	Gametes tested	Inversions	Percentage
600 r						
Series 1*	254	1	0.4	254	0	0.0
2400 r						
Series 2*	91	2	2.2	91	1	1.1
1500 r						
Series 3a†	185	2	1.1	185	0	0.0
Series 3b	116	1	0.9 } 1.0	116	1	0.9 } 0.3
2000 r						
Series 4‡	138	11	8.0	166	3	1.8
Total	784	17	2.2	812	5	0.6

* In Series 1 and 2, *y*^{2} *v f* ♂♂ were mated to + ♀♀, and the inseminated ♀♀ were then irradiated and their progeny examined for crossing over within the two marked regions of the X chromosome totalling 56.7 units in length.

† In Series 3, *al Sp b L*^{34}*/Cy*; *Ly Dl Pr/In(3LR)Cx*, *D* ♂♂ were mated to *pr cn*; *by*; *ci ey*^{R} ♀♀, and the inseminated ♀♀ were then X-rayed. F_1 ♀♀ that were Sternopleural and Lobe in phenotype were back-crossed to *pr cn*; *by*; *ci ey*^{R} ♂♂ in Series 3a, for a test of crossing over within the three marked regions of chromosome 2 totalling 50.0 units in length. Similarly, F_1 ♀♀ that were Curly Lyra Delta Prickly in phenotype were crossed to + ♂♂ in Series 3b, for a test of crossing over within the two marked regions of chromosome 3 totalling 49.5 units in length.

‡ In Series 4, Oregon-R wild-type ♂♂ or ♀♀ were X-rayed and mated respectively to *al b c sp*^{2} ♀♀ or ♂♂. The F_1 ♀♀ were test-crossed to *al b c sp*^{2} ♂♂ for a test of crossing over within the three marked regions of chromosome 2 totalling 107.0 units.

genetic tests for inversions induced by X-rays have been made, and while the data remain scanty, they indicate the probable correctness of the foregoing reasoning. In all series the progeny was limited to the first 5 days after treatment of the parents. Table 6 presents the results. The experiments are not directly comparable. In the first two series inversions were looked for in the X chromosome; in series 3 in both chromosomes 2 and 3; but in the fourth series, for which I am indebted to MR. WILLIAM RAPPAPORT, only in chromosome 2. More important, different lengths of the chromosomes were covered in the several tests, so that only in the fourth series, where the entire chromosome 2 was checked, could all the inversions induced in that chromosome be detected. Exact comparisons between frequencies in spermatozoa and oocytes must therefore be confined to individual series, but this is not important to the conclusions, since all the tests show that inversions are about three to four times as commonly induced in spermatozoa as in mature oocytes. The ratio of inversions induced in the female germ cells to those induced in the male is therefore considerably larger and consequently nearer equality than the ratio for induced translocations.

DISCUSSION

The experiments reported above reveal that in oocytes of Drosophila there is a relatively higher frequency of inversions than of translocations, the frequency of

each type of aberration in the spermatozoa being used as the respective basis of comparison. In other words, the ratio inv. ♀♀/inv. ♂♂ greatly exceeds the ratio trans. ♀♀/trans. ♂♂. The simplest explanation of this relation is that the non-random factor responsible in spermatozoa for the excessive yield of paracentric inversions, in comparison to pericentric inversions and translocations (see CATCHESIDE 1948, p. 284), is considerably strengthened in the oocytes. This factor is supposed to be the relation between the proximity of breaks to one another and the probability that the broken ends will join. It might even be suspected from the present data that the frequency of chromosome breaks induced by ionizing radiation is no lower in oocytes than in spermatozoa; but that in the oocytes most breaks undergo restitution and thus escape detection. This hypothesis is readily testable, by comparing the frequencies in oocytes and spermatozoa of very small deficiencies. These are known to increase linearly with the radiation dose, and are thus in most cases the effects of single "hits". In such cases the two breaks produced are so very close together that the proximity factor should be virtually the same in the mature male and female germ cells. In the next paper of this series the results of such experiments will be presented.

General discussion of the relation of chromosome breakage and rearrangement to cellular conditions, and in particular to the state of the chromosomes at the time of irradiation, will be deferred until the data on the induction of small deficiencies and recessive lethals have been presented. It is sufficient at present to point out that the Drosophila data reported here agree very well with the findings of BOZEMAN and METZ (1949) on Sciara and those of A. R. WHITING (1945) on Habrobracon. In the oocytes of all three of these insects, sensitivity is apparently minimal until the meiotic prophase begins. The sensitivity then rises rapidly until it reaches a maximum in metaphase (where meiosis halts in Habrobracon and Drosophila) or anaphase (where meiosis halts in Sciara). In Drosophila, the treatment of particular stages of the meiotic prophase and metaphase in oogenesis cannot at present be identified; but there is the same general shift from insensitivity to sensitivity that probably corresponds to the progression from preprophase to metaphase.

On the other hand, even in metaphase the frequency of translocations induced in female germ cells in both Sciara and Drosophila is practically zero, although in Drosophila infrared radiation appears to magnify somewhat the probability of obtaining interchromosomal rearrangements after X-ray treatment. This finding, like the difference between the ♀/♂ inversion-translocation ratios, points to the probability that it is not so much a difference in frequency of chromosome breakage that distinguishes oocytes from spermatozoa as it is a difference in the probability of obtaining a recombination rather than a restitution of breaks.

SUMMARY

1. In series in which spermatozoa and oocytes were irradiated with identical doses of X-rays, mainly 2000 r units, only 1 translocation in 2599 oocytes was obtained, in comparison to 150 translocations in 2387 spermatozoa.

2. With supplementary near infrared radiation, no statistically significant increase in the frequency of genetically detected translocations induced by X-rays in spermatozoa was found, either when the infrared radiation was given before the X-ray

treatment or when it was given afterwards. In oocytes, 6 translocations were found in 1534 oocytes following a supplementary infrared posttreatment, and only 1 translocation in 678 oocytes following X-ray treatment alone. The difference, although not statistically significant, is suggestive.

3. Chromosomes 2 and 3 are involved in translocations equally often; the X chromosome, less than half as frequently as either major autosome; the Y chromosome, somewhat less frequently than the X; and chromosome 4, about one tenth as often as the major autosomes.

4. In spermatozoa, the frequency of translocations produced in the progeny derived from spermatozoa utilized for fertilization 8 to 11 days after treatment was found to be almost twice as great as the frequency in either the first brood (days 1–4) or the second brood (days 5–7). All the translocations found in treated oocytes were in the first brood, i.e., where induced in the most mature oocytes.

5. Inversions (detected genetically) are induced by X-rays about 3 to 4 times as frequently in spermatozoa as in oocytes. The ratio inv. ♀/inv. ♂ is 40 to 50 times as great as the ratio trans. ♀/trans. ♂. This suggests that the frequency of chromosome breakage in oocytes is little different from that in spermatozoa, and that the difference in the frequency of rearrangements results from a difference in the probability of obtaining recombinations in accordance with the proximity of the breaks.

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THE EFFECT OF OXYGEN AND OF HYDROGEN PEROXIDE ON THE ACTION OF A SPECIFIC GENE AND ON TUMOR INDUCTION IN DROSOPHILA MELANOGASTER[1]

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IT IS now well established that irradiation with X-rays produces the highly oxidizing OH and HO_2 radicals, as well as H_2O_2, in aqueous media containing dissolved molecular oxygen (see GRAY 1953). It is also generally agreed that hydrogen peroxide represents at least one of the agents responsible for the effects of radiation on biological systems (THODAY and READ 1949; FEINSTEIN *et al.* 1950; GILES and BEATTY 1950; COLE *et al.* 1952). The amount of hydrogen peroxide produced depends upon the concentration of oxygen present at the time of irradiation with X-rays.

The effect of the X-rays upon the suppressor-erupt system in *Drosophila melanogaster* is to block the action of this specific suppressor gene and thus to allow the mutant gene *erupt*, which in most strains is present but suppressed, to manifest itself (GLASS 1944; GLASS and PLAINE 1950). The erupt mutation, when its suppressor gene is inhibited, produces in the eyes of the adult flies abnormalities which are classified as extreme or weak erupt, depending on the degree of manifestation. A high incidence of melanotic tumors is likewise induced by the treatment of embryos with X-rays. Irradiation of embryos of the suppressor-erupt mutant and Oregon-R wild-type strains in various concentrations of oxygen produces, with increasing oxygen concentration, an increased incidence of tumors in the larvae and an increased frequency of erupt eyes in the imagoes (GLASS and PLAINE 1952; PLAINE and GLASS 1952). If a part of the X-ray effect on the action of the suppressor-erupt gene, and on the induction of melanomas, were mediated through the action of hydrogen peroxide, then it should be possible to demonstrate an effect of hydrogen peroxide itself, provided this agent can enter the embryo.

It has already been shown that 10-minute exposures of 10-hr.-old embryos of the suppressor-erupt strain to various concentrations of oxygen have in themselves no apparent effect on the manifestation of erupt, although such treatments do influence the induction of melanotic tumors (PLAINE and GLASS 1952). This problem has been more thoroughly investigated in the present work.

INFLUENCE OF HYDROGEN PEROXIDE AND IRRADIATED WATER UPON THE INCIDENCE OF MELANOTIC TUMORS AND ERUPT EYES

Materials and methods

Eggs of the suppressor-erupt strain (*Su-er bw; st er*) were collected over a 2-hour interval and were maintained at 25°C for ten hours, at the end of which time they

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were dechorionated by the procedure of HILL (cf. SONNENBLICK 1950). The eggs were then washed several times with distilled water. The water was removed by filtration, leaving the completely dechorionated eggs well dispersed on the filter paper. This treatment, even together with the relatively rough handling, kills few if any embryos and in no way affects the incidence of tumors or erupt eyes. The entire process takes less than ten minutes.

The outlet of the Buchner funnel was stoppered, and the desired concentration of hydrogen perioxide solution was added until it fully covered the filter paper and eggs. Following treatment, the funnel was opened and the contents were flushed several times with distilled water in order to remove all traces of hydrogen peroxide. The filter paper was then placed in a 4-inch petri plate, and after the eggs had hatched, dried brewers' yeast was added (PLAINE 1952). Tumors were counted in the late third instar larvae, which were subsequently placed in vials to await eclosion, at which time the adults were classified for erupt.

Results

Two concentrations of hydrogen peroxide were used, and each series comprised at least two replications, which in all cases were found not to differ statistically. An exposure of 10-hr.-old embryos to three percent H_2O_2 for ten minutes killed many individuals, but a tumor incidence of only 7.25 percent was obtained. This is, nevertheless, significantly higher than the control value. In spite of the high mortality in early stages, 90 percent of the individuals that reached pupation eclosed. The frequency of erupt in these flies was 14.3 percent, which is likewise significantly higher than the control value. On the basis of these results it seemed desirable to use a stronger concentration for a shorter length of exposure, in the hope that the agent might prove more effective before becoming too toxic. Ten-hour-old embryos were, therefore, exposed to six percent H_2O_2 for five minutes.

This treatment proved even more toxic, for, in addition to the early killing, only 13 percent of the pupae eclosed. The data in table 1 show, however, that the six percent solution was no more effective in inducing tumor formation than the three percent solution. Table 2 shows the effects of the corresponding treatments on erupt eyes. Because of the smaller number of adults surviving after treatment with the higher concentration, the seeming increase in the frequency of total erupt flies for the six percent treatment is not significantly different from that for the three percent concentration. Consequently, it may be tentatively concluded that a maximal effect from externally applied H_2O_2 has been obtained, and that higher concentrations or increased lengths of exposure would merely increase the general toxicity of the agent without further affecting the incidence of either phenomenon.

EVANS (1947) found that heavily irradiated water had deleterious effects on Arbacia sperm placed in it. From extended studies using irradiated media, chemical tests, H_2O_2 solutions, and catalase extracts, he concluded that the chief, if not the only, agent responsible for the various effects of irradiated water was hydrogen peroxide. Likewise, KIMBALL and GAITHER (1952) have reported that irradiated medium, in which the accumulation of H_2O_2 was not prevented, produced death in some para-

TABLE 1

Comparison of effects of various treatments on the induction of melanotic tumors in Drosophila melanogaster (Stock: *Su-er; bw st er*)

Treatment‡	No. of series	Total	No. with tumors	Tumor incidence	Total no. tumors	Av. tumors per larva
1000 r units in air	3	1400	1144	81.71 ± 1.03§	1487	1.062
3% H_2O_2 (exp. 10 min.)	2	800	58	7.25 { 9.94*, ¶ / 5.09 }	84	.105
6% H_2O_2 (exp. 5 min.)	1	400	28	7.00 { 10.96† / 4.11 }	31	.078
O_2 saturated H_2O X-rayed with 10,000 r (exp. 15 min.)	1	400	14	3.50 { 6.60 / 1.57 }	16	.040
Control	4	1800	79	4.38 { 5.79 / 3.23 }	89	.049

* Significant from untreated controls at the 1% level.

† Significant at the 5% level.

‡ All treatments were at embryonic age 10 ± 1 hr. Length of exposure to each agent is given in parenthesis.

§ The ± values are standard errors.

¶ Bracketed figures are confidence limits at the 1% level of significance (STEVENS 1942).

TABLE 2

Comparison of effects of various treatments on the production of erupt eyes in Drosophila melanogaster (Stock: *Su-er bw; st er*)

Treatment*	No. of series	Total	Phenotype of eyes, %		
			Normal	Extreme erupt	Total erupt
1000 r units in air	3	435	8.5	83.7 ± 1.8†	91.5 ± 1.3
3% H_2O_2 (exp. 10 min.)	2	721	85.7	1.2 { 2.7‡ / 0.4 }	14.3 ± 1.6
6% H_2O_2 (exp. 5 min.)	1	49	79.6	2.0 { 14.3 / 0.0 }	20.4 ± 5.7
O_2 saturated H_2O X-rayed with 10,000 r (exp. 15 min.)	1	354	96.3	0.0 { 1.5 / 0.0 }	3.7 { 7.1 / 1.6 }
Control	1	500	90.8	0.0 { 1.1 / 0.0 }	9.2 { 13.0 / 6.2 }

* All treatments were at embryonic age 10 ± 1 hr. Length of exposure to each agent is given in parenthesis.

† The ± values are standard errors.

‡ Bracketed figures are confidence limits at the 1% level of significance.

mecia added to the medium after the irradiation, and delay in division in others. Both effects were roughly equivalent to those produced when H_2O_2 was added directly.

To test for a similar effect in the present study, dechorionated 10-hour-old embryos were immersed for 15 minutes in water which had previously been saturated with

pure oxygen while being irradiated with 10,000 r units of X-rays. The embryos were immersed as soon as the dose was delivered. As shown in tables 1 and 2, this treatment had no effect on either phenomenon.

INFLUENCE OF OXYGEN CONCENTRATION ALONE UPON THE INCIDENCE OF MELANOTIC TUMORS AND ERUPT EYES

Materials and methods

For a portion of this study two genetic stocks of *D. melanogaster* were used for comparison: (1) a suppressor-erupt stock, *Su-er bw; st er*, and (2) a wild-type stock, Oregon-R. The low frequency of affected individuals developing from X-rayed embryos of the wild-type stock contrasts strikingly with that previously found in the suppressor-erupt stock similarly treated (GLASS and PLAINE 1952; PLAINE and GLASS 1952). Hence, it seemed that the effect of oxygen concentration could best be determined by using two such contrasting stocks.

TABLE 3

The effects of exposing embryo; of two strains of Drosophila melanogaster to various concentrations of oxygen

Treatment	Total	No. with tumors	Tumor incidence	Total no. tumors	Av. tumors per larva
Suppressor-erupt stock					
1. 0% O_2 (N_2)	400	10	2.5 { 5.3 / 0.9 }	10	.025
2. 10% O_2 (10% O_2/90% N_2)	400	15	3.8 { 6.9 / 1.7 }	20	.050
3. 20% O_2 (air)	400	19	4.8 { 8.2 / 2.5 }	23	.058
4. 60% O_2 (60% O_2/40% N_2)	400	32	8.0 { 12.1 / 4.9 }	44	.110
5. 100% O_2	400	26	6.5 { 10.3 / 3.7 }	35	.088
$t_{1,5} = 2.66$ $P < 0.01$					
Oregon-R stock					
1. 0% O_2 (N_2)	400	3	0.8 { 2.7 / 0.1 }	3	.008
2. 10% O_2 (10% O_2/90% N_2)	400	5	1.3 { 3.5 / 0.3 }	5	.013
3. 20% O_2 (air)	400	8	2.0 { 4.6 / 0.6 }	10	.025
4. 60% O_2 (60% O_2/40% N_2)	400	15	3.8 { 6.9 / 1.7 }	15	.038
5. 100% O_2	400	19	4.8 { 8.2 / 2.5 }	23	.058
$\chi^2_{1,2,5} = 13.75$ d.f. = 2 $P = 0.001$					

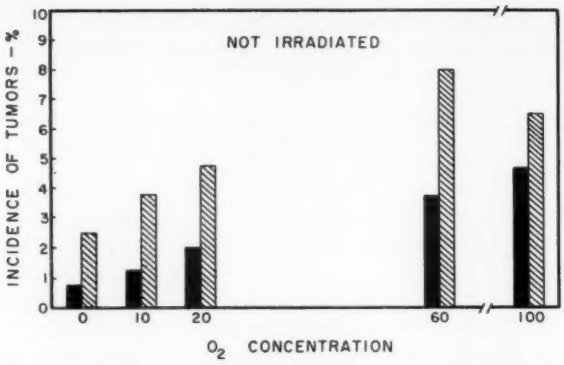

FIGURE 1. The incidence of melanotic tumors in third instar larvae following exposure of the embryos to various concentrations of oxygen for 10 minutes without irradiation. At each concentration the column to the left shows the data for the Oregon-R wild-type stock and the column to the right the data for the suppressor-erupt stock.

Eggs were collected over a 2-hour period and were exposed for ten minutes to oxygen of a given concentration (in N_2) at an embryonic age of 10 ± 1 hr. by methods previously described (GLASS and PLAINE 1952). After treatment, the same procedure was followed as that described above. The eggs and larvae were maintained at 25°C.

The gases used were as follows: pure N_2; 10 percent O_2/90 percent N_2; air (approximately 21 percent O_2/79 percent N_2); 60 percent O_2/40 percent N_2; pure O_2. The pure oxygen and nitrogen, and the gas mixtures, were from commercially supplied cylinders with low percentages of impurities.

Results

The incidence of tumors produced in five different series exposed respectively to N_2, 10% O_2, air, 60% O_2, and pure O_2 for ten minutes is given in table 3. Both the Oregon-R and suppressor-erupt strains clearly show an increased incidence of tumors with increasing oxygen concentration. This increase appears linear for the concentrations from 0 percent to 20 percent oxygen for both strains. At the 60 percent oxygen concentration the tumor incidence in the suppressor-erupt strain is higher

TABLE 4

The effect of increasing exposure to pure oxygen on melanotic tumors without X-ray treatment in Drosophila melanogaster (Stock: *Su-er bw; st er*)

Treatment	No. of series	Total	No. with tumors	Tumor incidence	Total no. tumors	Av. tumors per larva
100% O_2 (exp. 10 min.)	1	400	26	$6.50\begin{cases}10.36\\3.73\end{cases}$	35	.087
100% O_2 (exp. 60 min.)	2	800	230	28.75 ± 1.60	360	.450
Control raised in air	4	1800	79	$4.38\begin{cases}5.79\\3.23\end{cases}$	89	.049

TABLE 5

The effect of increasing exposure to pure oxygen on erupt eyes without X-ray treatment in Drosophila melanogaster (Stock: *Su-er bw; st er*)

Treatment	No. of series	Total	Phenotype of eyes, %		
			Normal	Extreme erupt	Total erupt
100% O_2 (exp. 10 min.)	1	1364	94.6	0.1 {0.5 / 0.0}	5.4 {7.1 / 3.9}
100% O_2 (exp. 60 min.)	2	673	77.3	1.3 {2.9 / 0.4}	22.7 ± 1.6
Control raised in air	1	500	90.8	0.0 {1.1 / 0.0}	9.2 {13.0 / 6.2}

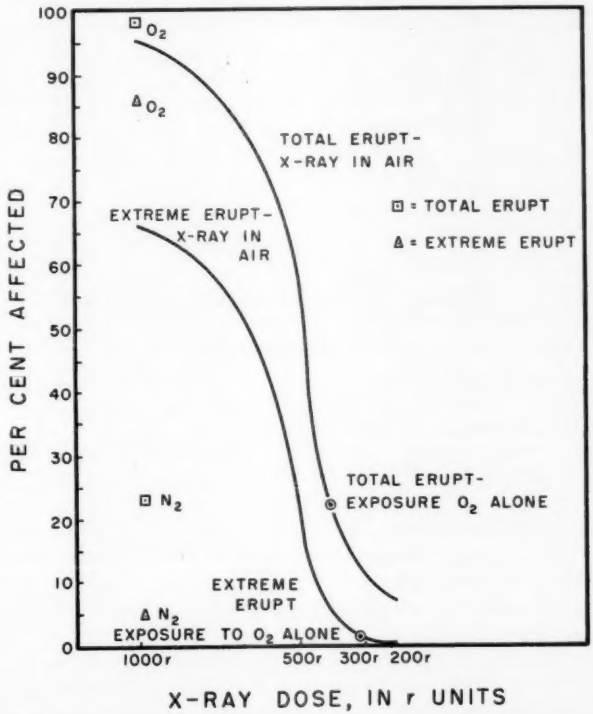

FIGURE 2. The percentage of suppressor-erupt flies manifesting erupt after being irradiated as embryos with the indicated doses of X-rays in air. The lower curve shows the percentages of extreme erupt and the upper curve the percentages of all types combined. ⊙ shows the respective frequencies of erupt produced by exposing embryos to pure O_2 for 60 minutes without X-ray treatment. Also included at the 1000 r dose are the percentages obtained by irradiating the embryos in pure N_2 or in pure O_2.

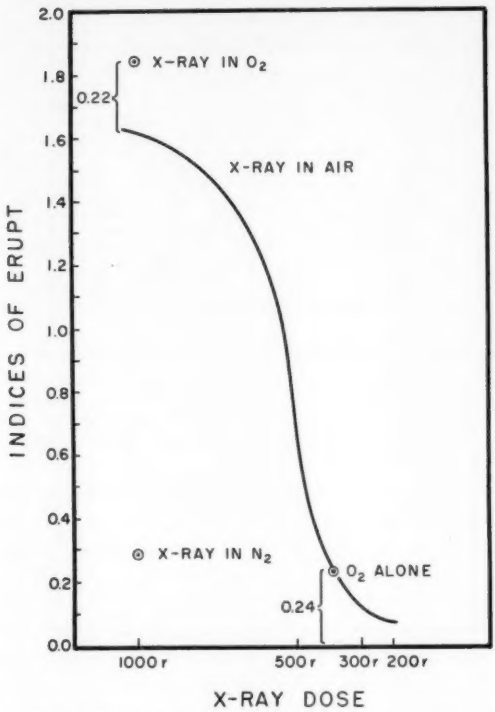

FIGURE 3. Change in the index of erupt manifested by suppressor-erupt flies after being irradiated as embryos with the indicated doses of X-rays in air. 0 = normal; 1 = weak erupt; 2 = extreme erupt. The figure also shows the indices of erupt produced by (1) exposure of embryos to pure O_2 alone; (2) exposure of embryos to pure O_2 during irradiation with 1000 r units; and (3) exposure of embryos to pure N_2 during irradiation with 1000 r units.

than at any other concentration. Perhaps, with respect to this strain at least, the oxygen effect is greatest at some concentration between pure oxygen and that found in air. However, this remains for the present in doubt, since none of the differences between any two consecutive concentrations is significant. The overall increase from 0 percent O_2 to 100 percent O_2 is statistically significant (table 3). A comparison of the tumorigenic effect of the various concentrations of oxygen is shown graphically in figure 1. Exposing 10-hr.-old embryos to these various concentrations of oxygen for ten minutes has, however, no effect on the manifestation of erupt in either strain of Drosophila.

To resolve the problem posed by the response to oxygen of the tumor effect but the lack of response to oxygen of the erupt effect, 10-hr.-old embryos of the suppressor-erupt stock, which is more sensitive than the Oregon-R stock, were exposed to pure oxygen for 60 minutes instead of 10 minutes as in all previous studies. The effect of

this 60-minute treatment on tumor induction is given in table 4, and its effect on the suppression of erupt in table 5. Increasing the length of exposure by a factor of six raises the incidence of tumors from 6.5 percent to 28.7 percent, this difference being highly significant. Corresponding to this increase, an inhibition of the action of the suppressor-erupt gene now becomes apparent. The frequency of erupt flies is increased from 5.4 percent to 22.7 percent. This difference is 10.2 times its standard error.

CONGER and FAIRCHILD (1951) have demonstrated an effect of oxygen on genetic material. By exposing Tradescantia pollen grains to pure oxygen, they obtained frequencies of chromosomal aberrations equivalent to those produced by about 600 r units of X-rays in air. In the present experiments, an X-ray dose of 300 to 400 r would be required to produce frequencies of erupt equal to those produced by simple exposure to O_2, both as regards the extreme manifestation of erupt and of all types, weak and extreme, combined. The data upon which this estimation is based are shown graphically in figures 2 and 3. In figure 2 the actual percentages of extreme erupt and of all types combined (total erupt), produced by irradiation in air at 4 different doses of X-rays, are plotted against the X-ray dose. The frequencies of extreme erupt and of total erupt, produced by exposure to pure oxygen alone, fall on the respective X-ray curves at points equivalent to doses of 300 to 400 r. In figure 3 the same comparisons are made using indices of erupt which were calculated by assigning the values 0, 1, and 2 to the classes normal, weak erupt, and extreme erupt, respectively. This figure indicates that the X-ray dose equivalent to a 60-minute exposure to O_2 is probably about 390 r units in air.

Of further significance (see below) are the results obtained when the respective values of erupt produced by irradiation with 1000 r units in pure nitrogen or in pure oxygen are added to the figures. Irradiation in nitrogen yields 5 percent extreme erupt and 23.5 percent total erupt; irradiation in oxygen yields 86 percent extreme erupt and 98 percent total erupt (fig. 2).

DISCUSSION

Although hydrogen peroxide is effective in producing melanotic tumors and erupt eyes, the treatment with H_2O_2 does not yield frequencies at all close to those produced by irradiation with X-rays (tables 1 and 2). These findings, however, do not invalidate the assumption that a large part of the effects of X-rays on this genetic system may be mediated through the peroxide formed during irradiation, since, during irradiation, a low concentration of peroxide would be formed in the immediate vicinity of the gene products and substrates. When hydrogen peroxide is supplied externally, however, it must penetrate the entire organism, thereby becoming extremely toxic, and perhaps losing some of its activity before reaching the vital points concerned with gene action.

Since the action of the suppressor-erupt gene is almost completely blocked when 1000 r units of X-rays are applied directly to the embryos, it was thought that the activation of water with 10,000 r units should be sufficient to produce a marked effect. EVANS *et al.* (1942), however, reported that doses from 2,000 r to 10,000 r were too low to produce detectable amounts of hydrogen peroxide in water and that positive results were obtained only by using doses around 100,000 r. Other conditions which

have been found to affect the amount of hydrogen peroxide produced in irradiated medium include (1) the oxygen content, (2) the pH, (3) the temperature, (4) the radiation intensity, and (5) the presence of substances which remove hydrogen peroxide (EVANS 1947). In the present study, distilled water saturated with oxygen was used and conditions (1), (2), and (5) may safely be ruled out. Nevertheless, the radiation intensity and the total dose given were quite low. Consequently, the failure to obtain positive results in the present case is probably due to errors in the experimental procedure. This conclusion is further substantiated by the fact that this treatment had no effect on the mortality or viability of the treated individuals.

The fact that simply exposing embryos to an increased oxygen concentration for ten minutes was able to induce tumors but not to produce erupt eyes raises an interesting problem. Although the frequency of manifestation of erupt in flies that were X-rayed in air (92%) is somewhat higher than the incidence of tumors in the larvae (82%), the incidence of erupt diminishes much more rapidly (24%) with decreasing oxygen tension than does the incidence of tumors (57%) (PLAINE and GLASS 1952). This may mean simply a difference in the thresholds of the two effects. That relation would not eliminate the similarity in the dependence of both phenomena upon the oxygen concentration at the time of irradiation. That is to say, both phenomena show a direct linear proportionality to the oxygen concentration at the time of X-ray treatment, from an oxygen concentration of 0 percent to one of 21 percent. In both cases this dependence is clearly a function of the genetic constitution of the strain studied (GLASS and PLAINE 1952). The induction of tumors by different concentrations of oxygen, without irradiation, likewise illustrates these two points, namely, the linearity of the increase in incidence up to that concentration found in air, and the difference in response according to the genetic constitution of the strain. That the difference between the production of tumors and erupt eyes is probably one of threshold is further suggested by the fact that increased exposure of suppressor-erupt embryos to pure oxygen is effective in blocking the action of the suppressor gene and in producing erupt. The enhanced induction of melanomas, therefore, parallels the effects of the X-rays, and of oxygen, on the suppression of erupt at given oxygen tensions. PLAINE and GLASS (1952) suggested that radiation may produce its effect in inducing melanotic tumors by destroying some substance which normally acts as a suppressor of the uncontrolled growth, a substance that furthermore is under genetic control. A genetic analysis (ROGERS, unpub.) shows that there is indeed a suppressor-tumor genetic system involved and that its action is like that of the suppressor-erupt system. The X-ray and oxygen treatments, then, block the action of this suppressor system too, thereby allowing the tumors to develop.

GLASS and PLAINE (1952) have shown that the frequency of erupt produced by irradiation in pure oxygen (98%) is slightly but significantly higher than that produced by irradiation in air (92%). But the 75 percent increment in erupt obtained when the strain is irradiated in oxygen (98%) rather than simply exposed to pure oxygen (23%) is not as great as the 83 percent increment obtained when the strain is irradiated in air (92%) rather than simply raised in air (9%). The reason for this is quite evident. The frequency of erupt produced by irradiation in air with 1000 r units is already near the maximum (fig. 2; GLASS and PLAINE 1950), and it would not

be possible to measure an increase beyond this maximum response. Consequently, the effect produced by irradiation in pure oxygen might be considerably greater, in reality, than is apparent. The picture presented by figure 3 may seem to indicate that the effect produced by irradiation in pure oxygen is equivalent to that produced by X-ray treatment in air plus that produced by treatment with pure oxygen alone. It should be remembered, however, that the exposure to pure oxygen was for 60 minutes, whereas the irradiation in oxygen lasted for only 10 minutes. Hence, these two effects are not strictly additive.

Figure 2 shows the reductions in the respective frequencies of total erupt and of extreme erupt when the X-ray treatment is given in pure nitrogen. These frequencies are only slightly, though significantly, higher (10 to 15%) than those obtained after exposure to pure nitrogen, or to air, alone. In other words, the increment in the frequency of erupt produced by irradiation in nitrogen over that occurring after exposure to nitrogen without irradiation is very small. On the other hand, the increment in the frequency of erupt produced by irradiation in air over that following exposure to air alone is extremely large (83%). If nitrogen itself is entirely without effect in producing erupt, as seems to be the case, then the concentration of oxygen is the only significant variable when two exposures to the same dose of X-rays are compared (see HOLLAENDER, BAKER, and ANDERSON 1951). Since exposure to 21 percent oxygen (air) produces only 9 percent total erupt and exposure to X-rays alone (in the complete absence of oxygen) produces only 23.5 percent total erupt, whereas the combined X-ray treatment in air produces over 90 percent total erupt, it is evident that the effects of the two components, oxygen and X-rays, are more than additive, that is, are synergistic. This, together with the fact that exposure to pure oxygen, without any supplementary radiation treatment, significantly increases the frequency of erupt, makes it apparent that the radiogenic effect does consist of an enormous enhancement of the effect of oxygen tension alone, as was previously suggested (PLAINE and GLASS 1952).

The results obtained from irradiation in different concentrations of oxygen (GLASS and PLAINE 1952; PLAINE and GLASS 1952), exposure to solutions of hydrogen peroxide, and exposure to pure oxygen alone, suggest that X-rays may produce both types of abnormal growth, viz., melanotic tumors and erupt eyes, by producing from oxygen certain potent oxidizing agents analogous to and including hydrogen peroxide.

The major opposition to admitting the importance of the hydrogen peroxide produced during ionizing radiation is derived from a consideration of the almost universal presence in tissues of the enzyme catalase, which rapidly destroys hydrogen peroxide, and perhaps other oxidizing agents as well. EVANS (1947) found that the toxicity of irradiated water to *Arbacia punctulata* sperm was reduced by catalase. By irradiating paramecia in a medium in which the accumulation of hydrogen peroxide was prevented by catalase, KIMBALL and GAITHER (1952) showed that delay in division and death of the organisms were both considerably reduced. On the other hand, FEINSTEIN *et al.* (1950) have shown that hydrogen peroxide destroys liver catalase when injected into intact mice and that whole-body irradiation of mice with X-rays also brings about a sharp reduction in the catalase activity of the liver. From these results they concluded that, in addition to producing toxic hydrogen peroxide throughout

the body, ionizing radiations also severely damage the body's chief protection against the agent. Of considerable importance, therefore, are the reports by GREENSTEIN (1941, 1943) that the liver catalase activity of tumor-bearing rats and mice is considerably less than that of normal animals.

CHANCE (1949) has shown that the first reaction of catalase with hydrogen peroxide involves the formation of a catalase-H_2O_2 complex. This complex does not decompose spontaneously under ordinary circumstances, but is relatively stable unless a second reaction occurs between this complex and a donor molecule. In the absence of such a donor, therefore, the catalase would remain united in the complex and be incapable of further action. This might account for its destruction by the peroxide. As SPARROW (1951) has suggested, some agents may exert a mutagenic effect not by direct action alone, but also indirectly by inhibiting catalase, so as presumably to cause a further accumulation of organic peroxides. In the presence of a suitable donor, however, the second-order reaction occurs, resulting in (1) the coupled oxidation of the donor, (2) a release of catalase, (3) an accelerated decomposition of hydrogen peroxide, and (4) protection against the effects of ionizing radiation.

Some of the substances which act as antioxidants and which protect against radiation effects may accordingly serve as donors in the above system. Cysteine, glutathione (PATT *et al.* 1949, 1950), and thiourea (MOLE *et al.* 1950; LIMPEROS and MOSHER 1950) protect rats and mice against ionizing radiations. Tumor cells may be protected in the same way (STRAUBE *et al.* 1950). Bacteria are protected against the lethal action of X-rays by sodium formate, ethanol, and various glycols (HOLLAENDER, STAPLETON, and MARTIN 1951); and mice by sodium nitrite and by p-aminopropiophenone (COLE *et al.* 1952). Sodium formate, ethanol, and sodium nitrite have been shown to accelerate greatly the decomposition of the catalase-H_2O_2 complex (SUMNER and MYRBÄCK 1951; CHANCE and HERBERT 1950).

In all previously reported cases, in which the irradiation is applied to the living organism (see PATT *et al.* 1952), the protective substance must be present at the time of irradiation. In the erupt and tumor systems of Drosophila, however, the effect of the X-rays in blocking the action of the suppressor-erupt gene, and in producing melanotic tumors, is greatly reduced not only by cysteine present at the time of treatment, but also by cysteine which is not fed to the larvae until after the irradiation has been completed. The complete data on these results are reported fully in another paper (PLAINE 1955).

On account of the extreme sensitivity of the suppressor-erupt system, it is ideal for further studies of the nature of the induction of uncontrolled growth by ionizing radiations and other agents, and their dependence upon oxygen, of the nature and modes of action of substances that protect against the injury produced by these agents, and of the relation of gene action to all of these.

SUMMARY

When Drosophila embryos of the suppressor-erupt strain are treated with solutions of hydrogen peroxide, there is a significantly increased incidence of melanotic tumors in the 3rd instar larvae and an increased frequency of erupt eyes in the adult flies.

When the embryos are exposed for ten minutes to concentrations of 0% O_2, 10%

O_2, air, 60% O_2, and 100% O_2, without being X-rayed, the strain shows a small but significantly increased incidence of tumors with increasing oxygen tension. The same treatment applied to 10-hr.-old embryos of a wild-type strain gives similar results, but at lower levels of incidence. The frequency of erupt is not affected by so short an exposure to O_2, but exposures of suppressor-erupt embryos to pure oxygen for one hour significantly increases the frequency of erupt. The radiogenic effect may therefore be regarded as an enhancement of the effect of oxygen tension alone.

The results obtained when embryos are exposed to solutions of hydrogen peroxide or to pure oxygen without irradiation, together with those obtained when embryos are irradiated in different concentrations of oxygen, suggest that X-rays may produce both melanotic tumors and erupt eyes by the production from oxygen of certain potent oxidizing agents analogous to and including hydrogen peroxide.

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A COMPARATIVE STUDY OF INDUCED MUTATION IN THE OOCYTES AND SPERMATOZOA OF DROSOPHILA MELANOGASTER

I. Translocations and Inversions[1]

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THE investigation of mutation rates in Drosophila females has been strangely neglected by geneticists. Nearly all studies of the action of mutagenic agents have applied solely to the males. Yet obviously, estimates of the effects of radiation upon a population ought not to ignore the existence of females. If the rates in males and females were alike, no great difference in the ultimate conclusions would result; but, quite the contrary, in those few studies in which mutagenic agents have been applied to Drosophila females it has been found that the response is very much lower than that found in spermatozoa. Accordingly, it seemed highly desirable to make precise quantitative studies of the rates of mutation in Drosophila females, to complement those accumulated over the past 25 years, by numerous workers, for the Drosophila males.

It very early became evident in the course of the present studies (Glass 1939) that the difference between the sexes in mutation frequency was not identical for different types of mutational change. There was a virtual absence or striking shortage of certain categories of mutation in the females, alongside an equal or only moderately reduced frequency of other types of mutation. These differences offered the possibility of a critical analysis of the mutation process on a comparative basis, in a way not possible from the analysis of mutation data derived from males alone. Such a comparative study, it might be hoped, would throw light in particular on the vexing question of the nature of recessive lethal mutations—that one category of mutations which has provided by far the most extensive data regarding the nature and frequency of mutation available today, and which yet is by present criteria indubitably a composite of a variety of types of genetic change.

In Muller's original X-ray studies of induced mutation, which were carried out in 1926, females as well as males were X-rayed, and mutations were obtained in both sexes. The data, together with later results, were first published in the joint paper of Patterson and Muller (1930). Muller's data (Patterson and Muller 1930, table 3) for females and males given the same dose (t2) showed fewer total mutations obtained from treated females than from the males (8.35% vs. 18.5%); fewer lethals from the females than from the males (6.02% vs. 15.4%), and even fewer mutations associated with chromosomal aberrations ("sectional dislocations") from the females than from the males ($1/18$ vs. $4/12$). In the joint study, Patterson and Muller confirmed the latter result. They found 0 out of 18 or 20 lethals, semi-

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other that all deficiencies, no matter how small, are lethal when homozygous. The latter is probably true in the overwhelming majority of cases in *Drosophila melanogaster*, although not in other organisms, for in *D. melanogaster* the only known type of deficiency found to be viable when homozygous is a small deficiency of 8 bands or less, including the locus of yellow, at the tip of the X-chromosome (DEMEREC and HOOVER 1936; SCHULTZ and CATCHESIDE 1937). As to the former assumption, since it is supposed by some geneticists that *all* X-ray-induced mutations are deficiencies, grading from the cytologically visible into the invisible, the distinction between point mutations and deficiencies becomes purely one of operational definition. Those mutations which are viable when homozygous we call by definition point mutations, and those which show a cytologically detectable loss we define as deficiencies. The two criteria may lead to somewhat overlapping categories, but in Drosophila the overlap appears to be indeed slight. This type of experiment, however, being very laborious, has yielded data only extensive enough to support more convincing evidence. Hence the latter will be presented first.

In the experiment just referred to dominant mutations were also scored. The great majority of these were mutations that produced minute bristles, and were lethal when homozygous. Since previous genetic and cytological studies (see BRIDGES and BREHME 1944) have indicated that the great majority of so-called Minutes are deficiencies, and that most of them are small, presumably one-hit deficiencies, the possibility of using Minutes for a comparative study of chromosome breakage in oocytes and spermatozoa suggested itself. The preliminary results of this study have been previously presented in the form of an abstract (GLASS 1952).

MINUTES

Two obvious difficulties to the use of Minutes for a study of mutation rates must be surmounted if the results are to have reliability and validity. First, Minutes have a low viability and develop at a much slower rate than wild-type flies, and indeed slower than most mutant types. Second, Minutes differ in their degree of departure from the normal size and length of bristles, so that whereas some Minutes are readily recognizable, others can be classified only with uncertainty even by quite experienced Drosophila workers.

To overcome the first difficulty, it is necessary to remove all progeny from the cultures to be scored, at least every ten hours during the first days after hatching begins. By thus preventing the development of a second generation of flies in the culture, the slow-growing Minutes have a chance to complete their development. It is advisable to enrich the medium with extra yeast, so that the Minute larvae are not subjected to competition with the more vigorous wild-type larvae for food. Over-population of cultures should also be avoided. If these precautions are observed, all the flies of an F_1 generation can be scored, even though the development of Minute individuals may be prolonged beyond 20 days from the inception of the culture.

The second difficulty requires, first, a degree of skill in detecting borderline Minutes on the part of the workers; second, and more important, a genetic test of all supposed Minute individuals by breeding them with wild-type flies. This runs into a new difficulty: a large proportion of the Minute mutants are sterile. In our experience,

however, the fertile individuals have always bred true as Minutes. That is to say, the observers may fail to score as mutations some borderline cases; but practically never is a genetically wild-type fly classified as Minute. It may therefore be reasonably assumed that the sterile flies tested were also genuine Minutes. Different groups of workers may be expected to exhibit different cut-off points in distinguishing Minute from wild-type flies; and the same group of observers, with increasing experience, will find more verifiable Minutes in similar numbers of the offspring of identically treated flies. The absolute numbers of Minutes detected therefore vary from experiment to experiment. Within experiments, however, a reliable comparison of the frequencies of Minutes induced at different doses may be obtained; and the same observers, once trained, show a high degree of consistency from experiment to experiment. Indeed, the effect of this subjective failure to detect Minutes is statistically comparable to the failure to examine all cultures to the emergence of the very last fly. Although the absolute rates are thereby altered, the comparison of the relative mutation rates in oocytes and spermatozoa is not affected.

Table 1 presents the results of the first two series of experiments in which Minutes were scored. In these experiments the treated individuals were transferred to fresh cultures after the fourth day, and again after the seventh day. Thus three broods were obtained, including respectively days 1 through 4, days 5 through 7, and days 8 through 11 after treatment. The frequencies of mutations were very similar in the first and second broods from both treated males and treated females. The third brood from the treated males showed about a doubling of the frequencies found in the first two broods; and the frequencies for the third brood from the treated females showed

TABLE 1

Minutes induced by X-rays in male and female germ cells of D. melanogaster, Oregon-R Wild-Type

	♂♂					
X-ray dose	3250 r			4000 r		
	N.*	M.†	%	N.	M.	%
Brood A (days 1–4)	1720	14	0.82	535	5	0.94
Brood B (days 5–7)	1787	11	0.62	474	4	0.85
Brood C (days 8–11)	944	15	1.59	272	4	1.47
Total	4451	40	0.90	1281	13	1.01
	♀♀					
X-ray dose	3250 r			4000 r		
	N.	M.	%	N.	M.	%
Brood A (days 1–4)	1448	14	0.97	876	17	1.94
Brood B (days 5–7)	3176	28	0.88	2243	39	1.74
Brood C (days 8–11)	3556	1	0.03	2563	6	0.23
Total (excl. oogonia)	4624	42	0.91	3119	56	1.80

* N., individuals examined.

† M., total no. Minutes.

a very striking drop. These changes are accordingly attributable to an increased frequency in treated immature male germ cells (probably spermatocytes), and a decreased frequency in treated oogonia. These shifts in frequency agree with those reported for induced translocations in the first paper of this series.

Although the difference between the two X-ray doses administered was quite small, nevertheless the indication that Minutes can be scored with reasonable objectivity and that the frequency of induced Minutes varies in proportion to the administered dose led to more extensive experiments to determine the comparative frequency of Minutes in oocytes and spermatozoa treated with various doses of X-rays from 500 r units up to 4000 r units (60 kvp., 15 ma., about 160 r/min.). The results of six series are summarized in table 2. In some of these series the Canton-S wild-type strain was used, in others the Oregon-R. Inasmuch as the results obtained with the two stocks can be shown by the chi-square test not to be significantly different at any X-ray dosage, it seems valid to combine them. In these series the parents were removed from the cultures after 5 days, so that the comparison is between mature spermatozoa and mature oocytes. In these experiments it was found better to raise the progenies of the irradiated flies in bottles rather than in vials, even though this meant the use of a number of parents of each sex per culture instead of single pairs. About 20 pairs were used per culture at the lower doses and up to 30 pairs at the higher doses. The average number of F_1 flies per bottle ranged from 638 in the control cultures to 248 in the 4000 r series, and varied inversely with the X-ray dose administered to the treated parent. Extra dry brewer's yeast was added to the bottle, usually about the fifth day after the culture was started, in order to supply optimal nutrition. Under these conditions, the Minute flies are relatively most abundant during the last days of emergence of the progeny. About half of them are found after the fifth day of each count, which may run through the 21st or 22nd day from the starting of the culture. In one series 23 percent of the Minutes were obtained after the ninth day of scoring (19th day of culture), although this was exceptional and usually less than 10 percent remain at this date. From these observations it follows that if the culture is discarded before all flies have eclosed, the count of Minutes will be considerably reduced.

Since the parents were left in the cultures for only 5 days, and since within that period only germ cells are utilized which were already mature when treated (see GLASS 1955, and below), there was no likelihood of any replication of induced Minutes. As for spontaneous Minutes, the frequency is in the first place very low: 0.04% in these

TABLE 2

Dominant Minute mutations induced in mature oocyles and spermatozoa at various doses of X-rays

	♀	♂
Untreated	22/27,335 (0.0805%)	
500 r	3/3235 (0.093%)	
1000 r	52/10,763 (0.484%)	48/9008 (0.532%)
2000 r	118/11,575 (1.02%)	77/8819 (0.873%)
3000 r	261/13,836 (1.89%)	173/8961 (1.93%)
4000 r	431/14,720 (2.93%)	247/9139 (2.705%)

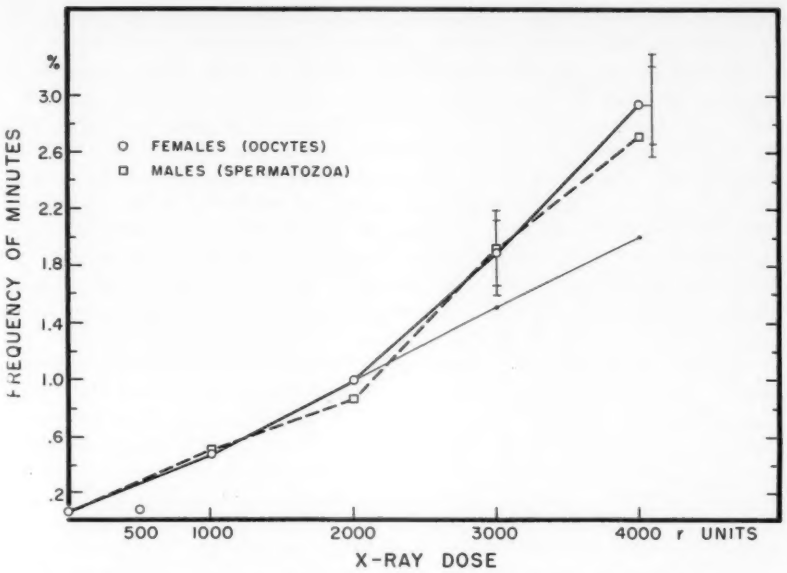

FIGURE 1. Comparative frequencies of Minutes induced by various doses of X-rays in Drosophila spermatozoa (squares) and oocytes (circles). Combined data from Canton-S and Oregon-R stocks. The fine line shows the expected increase at the two higher X-ray doses if the effect continued to be directly proportional to dosage. The vertical bars above and below the frequencies for the oocytes at 3000 r and 4000 r indicate the 5% and 1% limits of significance, by the inner and outer cross-bars respectively.

experiments (table 2, Control frequency ÷ 2). In the second place, these control Minutes never showed any clustering within cultures, such as might be expected if a Minute mutation were to occur prior to maturation in either germ line. The number of cultures with 0, 1, 2, or 3 Minutes, respectively, exhibited a remarkably good fit to the expected Poisson distribution. Consequently the probability of getting a spuriously high frequency of Minute mutation because of replication of an oogonial or spermatogonial mutation may be dismissed as negligible.

The data, graphed in figure 1, show that the frequencies of Minutes induced in the male and female germ cells are almost exactly equal at all doses from 1000 to 4000 r units. The dosage curve is strictly rectilinear up to a dose of 2000 r units. Above that point, it departs significantly from linearity, as may be seen (fig. 1) by comparing the measures of significance for the data from treated females at doses of 3000 r and 4000 r units with the straight line indicating the increase to be expected on the basis of a direct proportionality to dose. Above 2000 r units the curve rises at the 1.5 power of the dose. The dosage curves therefore imply that up to 2000 r most of the Minutes are induced by a single "hit" in each case, whereas above that dose an increasing proportion is produced by 2 hits on the chromosome per Minute. This agrees with

previous evidence that Minutes are in part associated with deficiencies cytologically observable, but for the most part are very small, 1-hit deficiencies.

As noted above, the absolute frequencies of Minutes produced by a given X-ray dose will obviously vary a great deal according to the conditions of the experiment. Little significance can be attached to such frequencies, except as expressing minimal rates for this type of mutation. The inability to compare different sets of data for Minutes is well illustrated (a) by a recent series conducted in this laboratory (not included in table 2), and (b) some data from the work of IVES, LEVINE, and YOST (1954, table 5). In our own series, controls for flies irradiated in oxygen were irradiated in air with a dose of 3000 r units. From the treated females 3.56% and from the treated males 3.06% of Minutes were obtained. That is, for both sexes taken together about 1.6 times as many Minutes were obtained as had previously been found at the same dose, in the more extensive series summed in table 2. Part of the increase may be attributed to greater skill in detecting borderline instances of Minute mutation, and to a more thorough testing of possible borderline cases. But it is also significant that through the ninth day of the scoring (18th day of culture) a frequency of only 2.60% of Minutes was obtained (both sexes combined: 199/7653). After that date scoring was continued for 3 days more, and yielded 97/1388 Minutes, or 6.99% for the final period of scoring. The high frequency of Minutes in this experimental series is therefore most probably due to unusually favorable culture conditions, of unknown nature, that led to the survival and detection of many Minutes that under the customary conditions would have perished.

IVES *et al.*, on the other hand, mated single treated males with 2 or 3 females in vial cultures, and discontinued the scoring after the 18th day (pers. commun.). At 3000 r they obtained only 1 percent of Minutes, a figure only slightly more than half the frequency for the same dose reported in the series summed in table 2 of this paper. A plot of their dosage curve indicates a linear increase up to 7500 r units, and a steeper rise thereafter. This may possibly mean that the Minutes which are due to small 1-hit deficiencies tend to be less delayed in development than the larger, 2-hit deficiencies. Hence the failure to score the entire progeny and to get the most severely delayed Minutes into the count would tend to reduce the exponential portion of the dosage curve and shift to a higher dose the point at which the departure from linearity becomes apparent. The attempt is being made to see whether a restriction of scoring to the Minutes eclosing in the earliest days of emergence of each culture will result in a completely linear dosage curve.

A random sample of the Minutes induced in the treated spermatozoa and oocytes was subjected to individual genetic analysis, in order to determine whether there is any marked difference in their association with gross chromosomal aberrations in the two groups. From the treated Oregon-R females 70 Minutes were tested, and from the treated males 39 were tested. None of the Minutes in the female series was associated with a translocation. In the Minutes from the male series 3 translocations were found. One of these did not even involve the chromosome bearing the Minute. Of the two others, the Minute was in one case readily separable from the translocation by crossing over. This leaves a single case in which a Minute was fairly closely associated with a translocation. Inversions were present in 3 out of 24 tested Minutes derived

from the treated spermatozoa. In two of these the Minute was separable by crossing over from the inversion, in one case because it was located in the center of a long inversion, in the other because it lay somewhat outside the limits of the inversion. In the third case the Minute was inseparable from the inversion, which blocked all crossing over to the right of the locus of black in chromosome 2. It remains quite possible that this particular Minute is also not at the locus of one of the breaks involved in the inversion. No definite inversions were found in the 43 Minutes derived from treated oocytes and subjected to a crossing over test. In one instance crossing over between roughoid and hairy in chromosome 3 was suppressed completely, and the Minute was inseparable from the said region; but the aberration was more probably a large deficiency than a small inversion. Two definite deficiencies for other loci were found to be associated with particular Minutes from treated spermatozoa in a total of 28 tested, and 5 were found in a total of 50 Minutes tested from treated oocytes. The proportion is essentially the same. The deficiencies in the oocytes included the loci of forked (X chromosome), curved (chromosome 2), curled (chromosome 3) twice, and cubitus interruptus (chromosome 4). The two deficiencies in the spermatozoa included the loci of curved and curled, respectively.

The distribution of the induced Minutes was somewhat different in the male and female series (table 3). In both sexes, over half of all Minutes are produced in chromosome 2. In both spermatozoa and oocytes, Minutes in the X chromosome are found with great rarity. In the female series, however, there seems to be a marked excess of Minutes in chromosome 4, although, in proportion to its size, this chromosome has more Minutes even in the series from treated males than one would expect. It might immediately be suspected that this excess is due to the presence of a considerable number of haplo-4 Minute individuals, produced by non-disjunction. However, when tested by crossing to cubitus interruptus (*ci*), none of 4 Minutes in chromosome 4 derived from treated spermatozoa and none of 7 derived from treated oocytes were found to be haplo-4. It is possible that in the female series some of the 8 Minutes that were in chromosome 4 and that were lost before this test could be completed were haplo-4; but the failure to detect any in the 7 tested cases indicates that the proportion is not likely to exceed one-third (5 percent level of significance).

In short, there is little evidence that the Minutes induced in either the oocytes or the spermatozoa are associated with gross chromosomal aberrations or haplo-4 deficiency. On the other hand, at least 7 to 10 percent of Minutes in both sexes are associated with fairly large deficiencies; and presumably the remainder are mostly, if not wholly, comprised of small, 1-hit deficiencies.

The fact that Minutes are equally frequent in X-ray-treated spermatozoa and

TABLE 3

Distribution of Minutes derived from treated oocytes and spermatozoa

	Chromosome				
	Total	X	2	3	4
♀	70	1 (1.4%)	37 (53%)	17 (24.3%)	15 (21.4%)
♂	37	0 (0.0%)	20 (54%)	13 (35%)	4 (11%)

oocytes at all doses indicates conclusively that the lack of translocations and other gross chromosomal aberrations in treated oocytes is not due to any shortage of chromosome breaks, but must reside in a lowered probability of recombination between breaks.

DEFICIENCIES

The earlier experiments offer evidence in support of the above conclusions. These experiments were planned to measure simultaneously in treated male and female germ cells the relative frequencies of (a) recessive mutations with visible effects, and (b) deficiencies. A second chromosome marked with the 8 recessive loci *al dp b pr cn c px sp* was combined with a chromosome from a male or female germ cell of the treated Oregon-R wild-type strain[2]. Any large deficiency would be manifested in an F_1 individual by the simultaneous appearance of two or more of the recessive markers. An F_1 fly showing the phenotype of a single marker gene might be attributable either to a small deficiency including the marked locus or to a point mutation at that locus in the treated chromosome. The alternatives were distinguished by crossing each such fly to a *Cy* sp^2/*al dp b pr cn c px sp* stock[2], and then selecting and breeding together the Curly non-speck males and virgin females from the progeny. Point mutations might be expected to be viable when homozygous and non-Curly flies of the mutant phenotype would appear; whereas if a deficiency had been induced in the original treated chromosome, only Curly flies would emerge in the progeny of the final cross. One other type of event might mimic a deficiency. If a recessive lethal mutation had been induced in the same chromosome as a visible mutation at one of the marked loci, no homozygous flies would appear in the final test progeny. The probability of this, from data that will be published in the third paper of this series, was approximately 10 percent for the X-ray dose used and the same genetic strain. It must be remembered, however, that recessive lethal mutations are themselves often due to deficiencies. The correction for this cause of the estimated frequency of induced deficiencies thus becomes almost negligible. (It should also be pointed out that when the original F_1 individual showing one of the marker phenotypes is a female, crossing over in its oocytes will complicate the task of testing. The tests described are best limited to F_1 mutant males; but to do so would reduce the numbers of mutants at the specific loci to such an extent that little information would be left. Consequently the effort must be made to test the mutant F_1 females in spite of the occurrence of crossing over. This type of test would be greatly improved if a marker chromosome that also bore adequate inversions to prevent crossing over were available.)

In the first experiment the effect of a supplementary infrared pretreatment was also tested. Table 4 presents these results, which revealed no significant difference between the effects of X-rays plus pretreatment with infrared and those of a treatment with X-rays only. As in the study of induced Minutes, described above, the

[2] In this paper genetic symbols for the following loci have been used: *y*, yellow, 1–0.0; sc^8 and sc^{8l}, scute alleles, 1–0+, associated with inversions of almost the entire X chromosome; *w*, white, 1–1.5; w^a, apricot, 1–1.5, allele of white; w^{m4}, white-mottled-4, in long inversion with left break to left of *w* and right break to right of *bb*; *fa*, facet, 1–3.0, deficiency for which produces Notch; *sn*, singed, 1–21.0; *B*, Bar, 1–57.0; *bb*, bobbed, 1–66.0. *al*, aristaless, 2–0.0; *dp*, dumpy, 2–13.0; *b*, black, 2–48.5; *pr*, purple, 2–54.5; *cn*, cinnabar, 2–57.5; *c*, curved, 2–75.5; *px*, plexus, 2–100.5; *sp* and sp^2, speck and speck-2, 2–107.0; *Cy*, Curly, associated with *Ins*(2*L* + 2*R*)*Cy*.

TABLE 4

Induction of recessives and deficiencies at 8 loci (Chrom. 2)

Series I. Oregon-R

	♂♂	♀♀	
		oocytes	oogonia
(a) Recessives			
2000r X-rays	5/4100 6/8162	0/3494 0/6805	0/1701 0/3644
2000r plus infrared pretreatment	1/4062 (0.07%)	0/3311	0/1943
4000r X-rays	1/1726 1/3354	0/1757 2/3927	0/1160 0/2820
4000r plus infrared pretreatment	0/1628 (0.03%)	2/2170 (0.05%)	0/1660
(b) Deficiencies (for 8 loci in chromosome 2) plus Minutes (probable deficiencies)			
2000r X-rays	10/4100 18/8162	3/3494 12/6805	0/1701 1/3644
2000r plus infrared pretreatment	8/4062 (0.22%)	9/3311 (0.18%)	1/1943 (0.03%)
4000r X-rays	6/1726 19/3354	9/1757 15/3927	0/1160 2/2820
4000r plus infrared pretreatment	13/1628 (0.57%)	6/1660 (0.38%)	2/1660 (0.07%)
(c) Total of recessive and/or deficiencies at specific loci, plus dominants (including Minutes)			
2000r X-rays	16/4100 33/8162	9/3494 20/6805	0/1701 2/3644
2000r plus infrared pretreatment	17/4062 (0.405%)	11/3311 (0.29%)	2/1943 (0.055%)
4000r X-rays	11/1726 27/3354	14/1757 26/3927	5/1160 9/2820
4000r plus infrared pretreatment	16/1628 (0.81%)	12/2170 (0.66%)	4/1660 (0.32%)

progenies of the treated males and females were divided into three successive broods (days 1–4, 5–7, and 8–11). Except for the very large drop in frequency in the third brood derived from the treated females, none of the differences were large enough to be statistically significant; and so in the table the three broods in the progenies of the treated males have been combined, and the first two broods in the progenies of the treated females have also been combined, as "oocytes," the third brood being distinguished as "oogonia." The data, although not sufficiently extensive for reliable conclusions, offer several interesting indications.

More recessive point mutations and deficiencies seem to be induced in spermatozoa than in the mature oocytes. If the equal numbers of Minutes found in the progenies of the treated males and females (10 in ♀; 11 in ♂) are deducted from the totals in Parts B and C of table 4, the relation becomes even clearer. At 2000 r, the respective percentages are 0.33 and 0.26; at 4000 r, 0.66 and 0.46. The differences are of course not statistically significant, but the fact that the doubling in frequency with doubling of the dose is virtually exact in both sexes, together with the agreement as to the difference between sexes at both doses of X-rays, carries some weight. This impression is strengthened by the further results of the series given a dose of 3250 r units, the Minutes from which were reported in table 1. The data are given in table 5, and again show that more recessive point mutations and deficiencies were induced in the spermatozoa than in the oocytes, although the absolute values fall somewhat below those found after the 2000 r dose in the first experiment (table 4).

The second interesting relationship, apparent in both table 4 and table 5, is the

TABLE 5

The comparative frequencies of point mutations and deficiencies involving 8 specific loci in Chromosome 2, induced by 3250 r units of X-rays, in male and female germ cells of D. melanogaster, Strain Oregon-R Wild-Type

	♂♂	♀♀	
		oocytes	oogonia
recessives	4/4451 (0.09%)	3/4624 (0.06%)	1/3556
deficiencies	7/4451 (0.16%)	4/4624 (0.09%)	
Total	11/4451 (0.25%)	7/4624 (0.15%)	1/3556 (0.03%)

greater number of deficiencies for specific loci in comparison with "visible" point mutations at those same loci. Again excluding Minutes, there were 6 recessive point mutations to 22 deficiencies at 2000 r. At 3250 r, there were 7 recessive point mutations and 11 deficiencies. At 4000 r, there were 3 recessive point mutations compared to 21 deficiencies. The trend is apparent in the oocytes as well as in the spermatozoa. Altogether, there were 3.3 times as many deficiencies recorded. Some of these, as has already been pointed out, might be cases where a recessive lethal mutation had been induced in the same chromosome as the recessive visible; but even if a correction of 10 percent be applied, there are still 3 times as many deficiencies as recessive visible mutations at the marked loci.

Of further interest is the relative number of large deficiencies that included more than one marked locus in each case. At 2000 r, there were 3 in the oocytes and 4 in the spermatozoa (0.044% and 0.049% respectively); at 3250 r, there were 4 large deficiencies, all from the treated spermatozoa (0.09%); at 4000 r, there were 4 in the oocytes (0.10%) and 2 in the spermatozoa (0.06%). Thus, of the total deficiencies, $17/_{54}$, or about one-third, were large deficiencies that included more than a single marker. The data are not extensive enough to compare the relative frequencies of the large deficiencies in oocytes and spermatozoa, but it is clear that they do occur in both types of germ cell, and there is no striking indication in this experiment that they are different in frequency. They would appear to behave about like the Minutes. As a matter of fact, one large deficiency included in this tabulation possessed an associated minute-bristle effect.

Recently the same type of experiment was repeated, at a dose of 1960 r units. Among the recessive point mutations and deficiencies definitely confirmed by breeding tests, there were 6 of the former and 19 of the latter. In the spermatozoa the ratio was 4:13 and in the oocytes 2:6. Here, too, as in the previous experiments, the ratio of recessive visible mutations to deficiencies that included the same marker loci is one-third. In this last series there was, however, quite a striking difference between the frequency in the male and female germ cells. The percentage in the former was 0.16%; in the latter, only 0.04%. If all apparent recessives and deficiencies for the 8 marker genes are included, the sterile as well as the tested, there were 53/10,328, or 0.51%, in the spermatozoa, and 44/20,117, or 0.22%, in the oocytes. This is still more than twice as many in the spermatozoa as in the oocytes. This difference is highly significant ($\chi^2 = 20.6$, d.f. $= 1$, $P \ll .001$).

DISCUSSION

MULLER (1954, pp. 492–496) and KAUFMANN (1954, p. 668) have summarized and discussed the problem of the induction of minute structural changes by ionizing radiations. The first person to recognize that very small structural rearrangements might constitute a special category distinct from gross chromosomal rearrangements was MULLER (1932), who made the observation that changes at nearby points tend to be associated with one another, and proposed that the effect of a single ionization might be able to spread so as to affect two nearby points in the chromosome. Evidence that in Drosophila this is the case was later accumulated by a systematic study of changes involving the scute locus (MULLER, PROKOFYEVA, and RAFFEL 1934, 1935), the yellow locus in the scute-8 inverted chromosome (MULLER, PROKOFYEVA-BELGOVSKAYA, and RAFFEL 1937; MULLER and BELGOVSKY 1937; RAFFEL 1938; BELGOVSKY 1939), and Notch deficiencies and reversions of Bar to normal eye (MULLER, MAKKI, and SIDKY 1939; MULLER 1940). From these observations it appeared clear that minute rearrangements in general exhibit a different dosage relationship than gross aberrations. The latter increase exponentially with dosage, at approximately a power of $1\frac{1}{2}$, whereas the very small deficiencies seemed to increase linearly with the dose.

A study of Notch deficiencies in the X chromosome, made by BAUER, DEMEREC, and KAUFMANN (1938), DEMEREC (1939), and SLIZYNSKA (1938), served as a basis for an analysis by DEMEREC and FANO (1941), who showed that deficiencies of more than about 15 salivary chromosome bands could be accounted for as being analogous to large-sized chromosomal aberrations, whereas there was a considerable excess of the short deficiencies over the number expected. These short deficiencies included all of those that arose spontaneously. The small deficiencies were accordingly interpreted as arising from a "single-event" or 1-hit process with a radius of action of about 600 Å. MULLER (1954) has pointed out several important qualifications of these conclusions. It seems very likely that "the spontaneous deletions occupied a more limited size range than the others . . . because most of them had arisen in interphase nuclei, in which the chromosomes are less coiled and in which there is therefore a greater physical distance between points a given distance apart along the chromosome, whereas the induced deletions had arisen from breaks induced in the tightly coiled chromosomes of spermatozoa and from unions occurring in chromosomes which were still somewhat more coiled than in ordinary interphase. As for the question why even those deletions which had been produced by radiation showed a frequency which declined with increasing size, the reason (in addition to that of the lower viability of larger deletions) might have been that ends derived from more distant breaks are less likely to come into contact with one another." The great shortage of all kinds of mutations in oogonia demonstrated in the present studies (GLASS 1952, 1955, and here) as well as in those of MULLER, VALENCIA, and VALENCIA (1949), would largely invalidate the first qualification, since it seems that few if any chromosomal aberrations of any kind originate in uncoiled chromosomes. The second suggestion is in complete agreement with the views advanced here and in the preceding paper (GLASS 1955) to explain, on the one hand, the equal frequency of Minutes in X-rayed spermatozoa and oocytes, and on the other hand, the increasing

rarity of inversions and translocations as the distance increases between the breaks that join.

MULLER (1940) discussed in some detail the cytological work of MARSHAK (1939) on Vicia, in which small chromosome fragments produced by irradiation with X-rays showed a linear frequency-dosage relation. If these fragments were in actuality interstitial rather than terminal, as MARSHAK thought them to be, and if they were really small enough, these data would confirm the data from Drosophila indicating that very small deletions can be produced by two breaks derived from a single ionization. Better evidence came from the work of RICK (1940) on Tradescantia. Chromosomes from irradiated microspores are frequently broken and give rise to chromosome fragments of very small size, averaging one micron in diameter. At low doses and low intensities of dose the frequency of these was found to increase as the first power of the dose; whereas at higher doses the curve became exponential. No sharp distinction between 1-hit and 2-hit aberrations could be made, but RICK estimated that almost half of the small deletions at the lowest doses were of the 1-hit type.

Evidence that disagreed with the previous findings came from the work of PANSCHIN, PANSCHINA, and PEYROU (1946). These colleagues of TIMOFÉEFF-RESSOVSKY undertook to retest the linearity with respect to dosage of the very smallest deficiencies by conducting tests of remarkable extent, conducted in Berlin during the last years of the war. At three doses (2000 r, 4000 r, 8000 r) they determined the frequency of appearance of white, Notch, bobbed, and all combinations of these characters, produced as a result of irradiating w^{m14}♂♂ and crossing them to *y w bb* ♀♀.[2] In this treated X chromosome, because of an inversion, the loci of *w*, *fa*, and *bb* lie very close together.[2] These workers therefore assumed that manifestations of one or more of the marker characters were due to tiny deficiencies, although it was recognized that some of the *w* mutations might be point mutations or position effects associated with gross chromosomal alterations. These very small deficiencies increased almost exactly as the square of the dose. MULLER (1954) has pointed out that because PANSCHIN *et al.* failed to use a region hemmed in to one side by the end of the chromosome and to the other by an adjacent viability gene, the loss of which would bring about death, it might be that a large proportion of the changes found and attributed to minute deletions were actually deficiencies of considerable extent or other gross aberrations. PANSCHIN *et al.* definitely state, however, that "die *zytogenetische Überprüfung* [ital. added] der besagten in der F_1 auftretenden Mutationen zeigte, dass die Mehrzahl der Mutationen kleine Deficiencies darstellt . . .". This statement destroys most of the force of MULLER'S criticism, although it remains, and probably will forever remain, unclear to just what extent the cytogenetic examination of the deletions produced in the experiments of PANSCHIN *et al.* were carried out. The use of the Notch phenotype as a visible indication of a small deficiency is also questioned by MULLER.

More recent studies by VALENCIA and MULLER (1951), unfortunately reported as yet only in an abstract, relate to more typical euchromatic regions of the X chromosome, and show that for deficiencies of more than 3 bands there is a random distribution of distances between breaks, but that the extremely minute deficiencies, of less than 4 bands, "although infrequent relatively to the total, occur much oftener

than they would on a random distribution of breaks and unions." (MULLER 1954, p. 496). If this is correct, probably the limits of size of the 1-hit deficiencies were estimated as too large by DEMEREC and FANO. A three or four-band limit (120 Å) seems more plausible for the spread of effect of a single ionization than a 15-band spread (600 Å), even in coiled chromosomes.

A recent study by LÜNING (1952, 1954) has utilized a method for determining the frequency of very small deficiencies that was first outlined by BELGOVSKY (1938, 1939). BELGOVSKY found that the excessively high frequency of recessive sex-linked lethals induced in the sc^8 or sc^{81} chromosomes was due to a high rate of very small deficiencies for the locus of yellow (*y*). LÜNING has X-rayed the sc^{81} *B Ins* w^a sc^8 (Muller-5) chromosome in males thereafter mated to *y w sn* females. Between doses of 960 r and 3840 r he found an increase of 7.5 times in offspring from the first brood (1–6 days) after treatment. These results, like those of PANSCHIN, PANSCHINA, and PEYROU (1946), thus indicate a logarithmic increase in the frequency of very small deficiencies with increasing dose of X-rays, but not an increase amounting to the square of the dose, as PANSCHIN *et al.* had reported.

The present studies on the induction by X-rays of Minute-bristle mutations offer a possibility of harmonizing the conclusions of the earlier studies with those of PANSCHIN *et al.* Up to 2000 r units, the dosage curves for the Minutes are strictly linear. Above that dose an exponential function becomes apparent. This appears to mean that at relatively low doses most of the Minutes, if they are indeed deficiencies, result from single ionizations that cause two breaks in the chromosome. At higher doses, larger deficiencies, caused by two breaks that result from separate ionizations, preponderate. It is interesting that this happens in the female germ cells as well as in the male, for that fact implies, since the dosage curves in the two sexes are identical, that there is no diminution in oocytes of the probability of reunions between increasingly distant breaks, as there is for inversions and translocations, so long as the breaks fall within the same arm of the chromosome. Perhaps the reduction in the relative frequency of inversions in oocytes, in comparison to their frequency in spermatozoa, applies only to pericentric inversions; and perhaps the paracentric ones occur as often as deficiencies of comparable length. At any rate, the conclusions already drawn clearly make it worth while to make a cytological study of the Minutes induced in male and female germ cells at low as well as high doses, so that it may be determined from a representative sample what proportion of Minutes is attributable to single ionizations.

The relation of these conclusions to the interpretation of X-ray-induced recessive lethals will be taken up in the following paper of this series.

SUMMARY

1. Dominant Minute-bristle mutations, which are attributable mainly if not exclusively to the production of chromosome deficiencies, and mostly to small deficiencies, are produced at X-ray doses from 1000 r units to 4000 r units at equal frequencies in spermatozoa and mature oocytes.

2. Up to 2000 r units, the increase with dose is rectilinear. Above 2000 r, the frequency of Minutes increases approximately as the 1.5 power of the dose. The shape

of the dosage curves may be interpreted as signifying that at the lower doses most of the Minutes result from single ionizations, whereas at higher doses an increasing proportion of Minutes is due to 2-hit effects.

3. In successive broods of the progenies from treated males and females, a doubling in the frequency of Minutes occurs in the third brood (8–11 days after treatment) derived from the treated males, and a very great drop in frequency occurs in the third brood from the treated females. These changes in frequency are in accord with those found in the case of induced translocations, and are attributed to the succession of treated spermatozoa by treated spermatocytes and of treated oocytes by treated oogonia.

4. The Minutes obtained were not associated with gross chromosomal rearrangements or with haplo-4 deficiency, except in a few possible cases, derived from treated males, in which translocations or inversions were present. It is very probable that most, if not all, of the Minutes are due to deficiencies. At least 7 to 10 percent are associated with fairly large deficiencies, the remainder presumably with very small ones.

5. The distribution of the Minutes with respect to particular chromosomes was not random in relation to the relative lengths of the chromosomes. Over one-half fell in chromosome 2, one-fourth to one-third in chromosome 3, 10 to 20 percent in chromosome 4; while Minutes in the X chromosome were rare, only 1 being found among 107 tested.

6. A study of point mutations and deficiencies induced at 8 selected loci in chromosome 2 also indicates that the chromosomes in oocytes are frequently broken by irradiation with X-rays. However, in this experiment more deficiencies were produced in the treated spermatozoa than in the oocytes, probably because this method of detection leads to a relatively larger number of big deficiencies. At least one-third of them were recognized as such.

7. The frequency of deficiencies for specific marked loci was about three times as great as the frequency of viable (point) mutations for those same loci. This applied to the oocytes as well as the spermatozoa.

8. There was no apparent effect of a supplementary infrared pretreatment upon the frequencies of X-ray-induced point mutations or deficiencies at marked loci, or of Minutes.

9. From the identity of the dosage curves for X-ray-induced Minutes in spermatozoa and in oocytes, and from the identification of the Minutes as being principally small deficiencies, it is concluded that chromosomes are broken by X-rays with equal frequency in mature oocytes and in spermatozoa. Consequently the almost complete failure of X-rays to induce translocations in oocytes, and the relative reduction of inversions to one-fourth the frequency obtained from identically treated spermatozoa, must be attributed to a probability of reunion between broken ends that in oocytes decreases more rapidly with the distance between breaks than it does in spermatozoa.

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